A STUDY ON THE PRODUCTION AND PROPERTIES OF SECRETORY IGA WITH PARTICULAR REFERENCE TO RECOVERY FROM AND RESISTANCE TO VIRAL INFECTIONS.

BY

SHEILA O. CAMERON, B.Sc.
(NEE CLARK)

being a thesis submitted for the Degree of Doctor of Philosophy in the University of Glasgow.

1983.

To My Parents and Alasdair

SUMMARY

The local and systemic immune responses to an inactivated, intranasal Influenza vaccine were evaluated in healthy volunteers found to have either no antibody or low titres of antibody to Influenza A/Victoria/3/75 (H3N2). A similar control group who received placebo (an aerosol with the same appearance and composition as the vaccine except that it did not contain virus) intranasally was included. Nine of twenty-two recipients and none of twenty-one placebo recipients developed circulating H-I antibody to the vaccine. However, six volunteers in each group developed a four-fold rise in local H-I antibody levels. Four vaccine recipients and five placebo recipients developed four-fold rises in local neutralising antibody levels. Four months after the intranasal administration of vaccine or placebo all subjects were challenged intranasally with an attenuated vaccine against the same virus. Five placebo recipients and four vaccine recipients subsequently developed circulating H-I antibody.

The interpretation of results was complicated by a natural outbreak of Influenza in the community at the same time as the trial. In the volunteers the inactivated vaccine showed a serological response. There was a slight protective effect against the challenge vaccine but not against natural infection. However, the timing of natural infection was unknown and it is likely that the vaccine had insufficient time to 'take'.

The response to natural infection is of longer duration than that induced by vaccination. However, antibody levels ($\geq$ 1:16) were maintained for the four month test period.

The criteria used for selection of volunteers was questioned and the recommendation was made that, in future, sIgA levels as well as serum H-I antibody levels should be measured prior to accepting a subject for a vaccine trial.

The predominant respiratory pathogens in a community were analysed during two winter periods and sIgA production during and following respiratory infection was evaluated. Selected subjects were examined over the two year period. These showed individual and variable patterns of sIgA production. There was no difference in sIgA production between patients suffering from repeated respiratory infections whether complicated by allergies or not when compared to the rest of the General Practice patients studied.

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PROPOSITUS

The aims of the study are :

Part 1. Vaccine Trial

1. to study the protective efficacy of an inactivated Influenza vaccine, administered by single dose aerosol. Four months after vaccination the degree of protection to be assessed by measuring immunity to challenge infection with a live, attenuated virus vaccine introduced intra-nasally, in the light of an evaluation of the volunteer's previous experience with Influenza virus infections.

2. to assess over the same period the persistence of local and serum antibodies elicited by vaccination against Influenza.

3. to evaluate the correlation between the level of local and serum antibodies and response to artificial infection.

Part 2. The Secretory Immune Response to Respiratory Infections.

1. to study secretory immunity following naturally acquired Influenza and other respiratory viral diseases during two winter periods.

2. to examine the dynamics of secretory antibody production in terms of time of initiation, variation in total duration and quantity of production both during infection with recognised bacterial and viral pathogens and particularly relating to previous history of respiratory infection. This latter to include possible malfunctions of the secretory system as may happen in chronic bronchitis and emphysema.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Our understanding of the aetiology and epidemiology of the respiratory and gastrointestinal tract diseases caused by viruses far exceeds our capacity to control or to alter the course of these illnesses. Effective immunoprophylaxis still represents a formidable challenge.

The mucosal surface of the respiratory tract is an extensive area that must be protected from penetration by pathogenic organisms and from toxic agents in the environment. Protection is mediated by a local immune system which is independent of systemic immunity (Bienenstock (1974); Tomasi (1976); Lamm (1976).) In contrast to serum in which IgG is the predominating immunoglobulin, secretions contain secretory IgA, an 11S dimer with secretory component (S.C) and J-chain forming a molecule ideally suited to the environment in which it is active. Protection occurs at the level of the mucous membrane by the neutralisation of viruses and control of bacterial proliferation by limiting mobility and adherence to epithelial cells (Walker and Isselbacher,(1977)).

IgA found in secretions differs from serum IgA and will be extensively reviewed in the following chapter. The role of monomeric serum IgA in the immunological defence system is poorly understood. Possible functions of serum IgA have been reviewed recently by Schuurman (1980) and will not be discussed further.

In order to augment the ideas reviewed here, which are only those relevant to my study, the reader is directed to several excellent reviews which give an historical consideration of the development of the concept of local immunity. (Tomasi and Grey (1972) and Heremans (1976)).

1.2 MOLECULAR STRUCTURE OF IgA

The human IgA monomer present in serum consists of two heavy polypeptide chains and the two light chains linked together by disulphide bridges. Antigenic analysis of IgA myeloma proteins have revealed two subclasses characterised by small antigenic differences in the constant part of the heavy chain. These subclasses are called IgA1 and IgA2. IgA2 differs strikingly from IgA1 in the hinge region. This leads to differences in susceptibility to digestion by proteolytic enzymes. For example, the protease derived from Streptococcus sanguis selectively cleaves IgA1 molecules whereas IgA2 is resistant to attack by this enzyme (Plaut (1978)). At present IgA1 is the only known substrate for the proteases produced by several species of bacteria. How protease production affects the pathogenicity of these organisms is an area stimulating much current interest. In normal serum about 90% of the total IgA belongs to the IgA1 subclass and about 10% to the IgA2 subclass.

Human serum IgA is almost completely monomeric with a small percentage of polymeric IgA which is composed of monomers linked by J-chain. In contrast the IgA of secretions is largely dimeric. It contains J-chain and a secretory component (S.C.) There are two proposed models for secretory IgA (figure 1).

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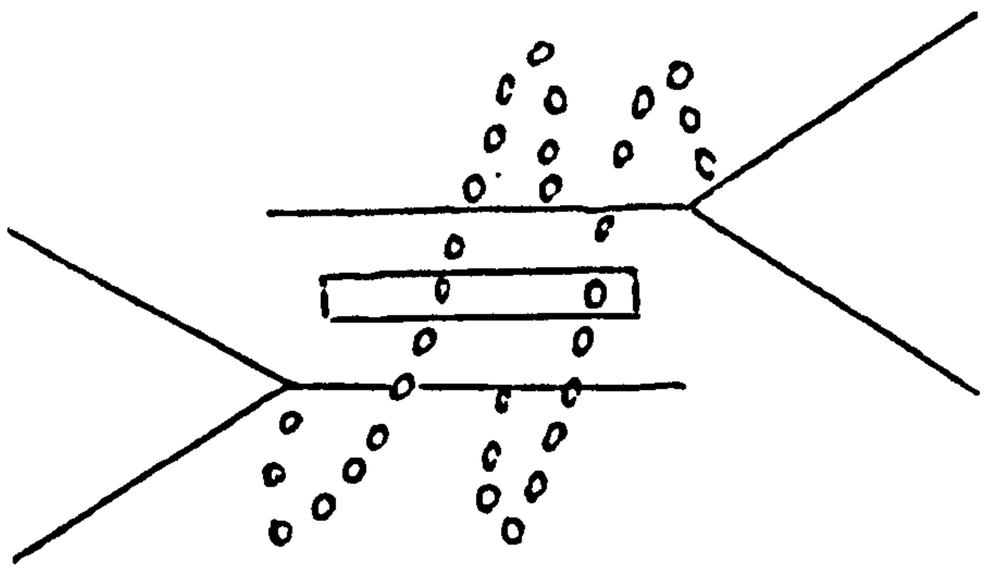
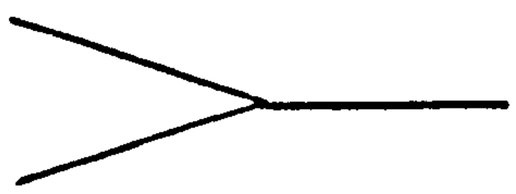




Monomer of IgA

J-chain

Secretory component (SC)

Figure 1. Proposed models for secretory IgA (Tomasi, 1972)

It is generally agreed that the J-chain is held in disulphide linkage with the carboxy terminal area of the heavy chains of the two IgA molecules which are, in turn, opposed by the tips of their Fc regions. The physical arrangement is brought about in the plasma cells which synthesise both IgA and J-chain. The evidence for the intracellular formation of dimers comes from studies of secretory IgA in human colostrum. It was shown that a single sIgA molecule contains either κ or λ light chains and mixed molecules are not present in appreciable quantities. This random reassociation of 7 S monomeric units in the interstitial region seems unlikely. It is strongly suggested that the J-chain, discovered by Halpern and Koshland (1970), is the mediator of polymerisation in both IgA and IgM (Parkhouse and Della Corte (1974)). IgA monomers polymerise in a ' linear' structure, different from the cylindrical structure of pentameric IgM (Hauptmans and Tomasi (1975)).

The linear structure has no restriction in the number of polymers per IgA molecule; this is, in turn, reflected by the existence of serum IgA myeloma proteins with a variable degree of polymerisation. (Roberts-Thomson et al (1970)).

Secretory component is present in secretory fluids, both free and bound to immunoglobulins (Brandtzaeg et al. (1970)). It is largely bound covalently to IgA but is only non covalently associated with IgM (Weiker and Underdown (1975)). Secretory component has a high glycine content which makes the molecule very flexible; it can, therefore, easily coil around two adjacent heavy chains in the IgA molecule. (Heremans (1974) and Mestecky (1978)).

With the use of Immunofluorescent techniques on intestinal tissue, it has been shown that SC is synthesised in the columnar epithelial cells lining the mucous membranes while J-chain containing IgA is synthesised by plasma cells in the lamina propria (Brandtzaeg and Balkien (1976)).

1.3 ORIGIN OF SECRETORY IgA (sIgA)

The lymphoblast precursor cells of the IgA producing plasma cells are probably derived from gut associated lymphoid tissue (G.A.L.T.) primarily Peyer's patches and are precommitted to IgA production before becoming distributed along the mucosal surface (Cebra et al. (1977)). These lymphoblasts belong to a sub-population of B-cells which bear no IgM but probably have IgA on their membranes (Craig and Cebra (1975)).

Immunisation attempts have shown that an antigen applied to the mucous membrane penetrates these membranes and encounters the B-lymphocytes. The plasma cells which result from B-lymphocyte stimulation produce IgA and this is actively transported through the epithelial cells to the surface of membrane where the original antigenic stimulation occurred.

Numerous examples clearly demonstrate the efficiency of this pathway of immunisation. For example, stimulation of one eye via the conjunctival sac led to the appearance of antibodies in tears from the immunised site but not from the other eye. (Centifanto et al. (1970)). Similarly the application of poliovirus antigen into the large intestine of individuals who have had double-barrelled colostomies induced sIgA antibodies restricted to the site of antigen application but failed to induce antibodies in other parts of the intestine, in glandular secretions or in nasal secretions. (Ogra et al. (1968)). These are only two examples of the experiments that are suggestive of an IgA-associated immune response that results from topical antigen application. Based on these results the concept of secretory IgA mediated local immunity was proposed. (Reviews: Tomasi (1976) and Heremans (1974)).

However, these experiments did not satisfactorily explain the presence of antibodies, naturally or artificially induced, in the secretions of glands that are remote from mucous surfaces such as the mammary glands or the salivary and lachrymal glands. These are connected to the mucous surface by ducts of various lengths. The wide range of sIgA antibodies found in these secretions added to the confusion. (Arnold et al. (1976)).

It has been established that IgA found in these secretions is produced by numerous plasma cells which are distributed in the parenchyma of the gland. (Heremans (1974)). Since IgA is the predominant secretory immunoglobulin and since it is actively transported in secretions, the mechanism of the sIgA mediated response in glandular secretions is open to question. If antigens that are present on a remote mucous surface are absorbed, enter the circulation and are carried to the lymphoid system of a gland where they induce an immune response, as demonstrated by the appearance of sIgA in the secretion, one would then expect a parallel serum response.

In addition,Montgomery et al. (1976) indicated that the presence of antibodies in sera of animals immunised systemically did not prevent a subsequent secretory response induced by local (oral) ingestion of antigens.

Thus a new pathway for the induction of the secretory immune response has been formulated from the findings of Montgomery et al (1974 and 1976) and Cebra's previous observations concerning the origin of IgA precursor cells and their homing patterns. (Craig and Cebra (1971) and Robertson and Cebra (1976)). According to this theory a population of lymphocytes from gut or bronchial associated lymphoid tissues (GALT and BALT) have the potential for seeding other tissues and differentiating into IgA producing plasma cells. (Craig et al. (1971) and Rudzik et al. (1975)).

These lymphocytes also carry the commitment to produce specific antibodies to antigens encountered in GALT and BALT. The mechanisms by which such lymphocytes are sensitised by antigen and how mature plasma cells subsequently home to the mucosal surfaces are not yet clearly established. Several theories have been proposed by Bienenstock (1978).

Oral immunisation studies have shown the appearance of antibodies to the ingested antigen in secretions of mammary (Montgomery et al (1974), salivary and lachrymal glands. (Mestecky (1978a)).

The available information concerning the origin of IgA producing plasma cells and the spectrum of IgA-associated antibodies found in external secretions suggest strongly that there are two pathways of stimulation for a secretory antibody immune response. The first is a local immune response induced by topical antigen application, the second mechanism of induction operates through the sensitisation of GALT and possibly BALT.

The latter pathway of stimulation leads to the appearance of specific IgA-associated antibodies in secretions of mammary, salivary and lachrymal glands(and perhaps other sites). This in turn suggests the existence of a common mucosal secretory system. (Figure 2).

The factors determining which type of secretory response is evoked are largely inknown. This is an area which needs further research. It offers fascinating possibilities in the field of vaccine development.

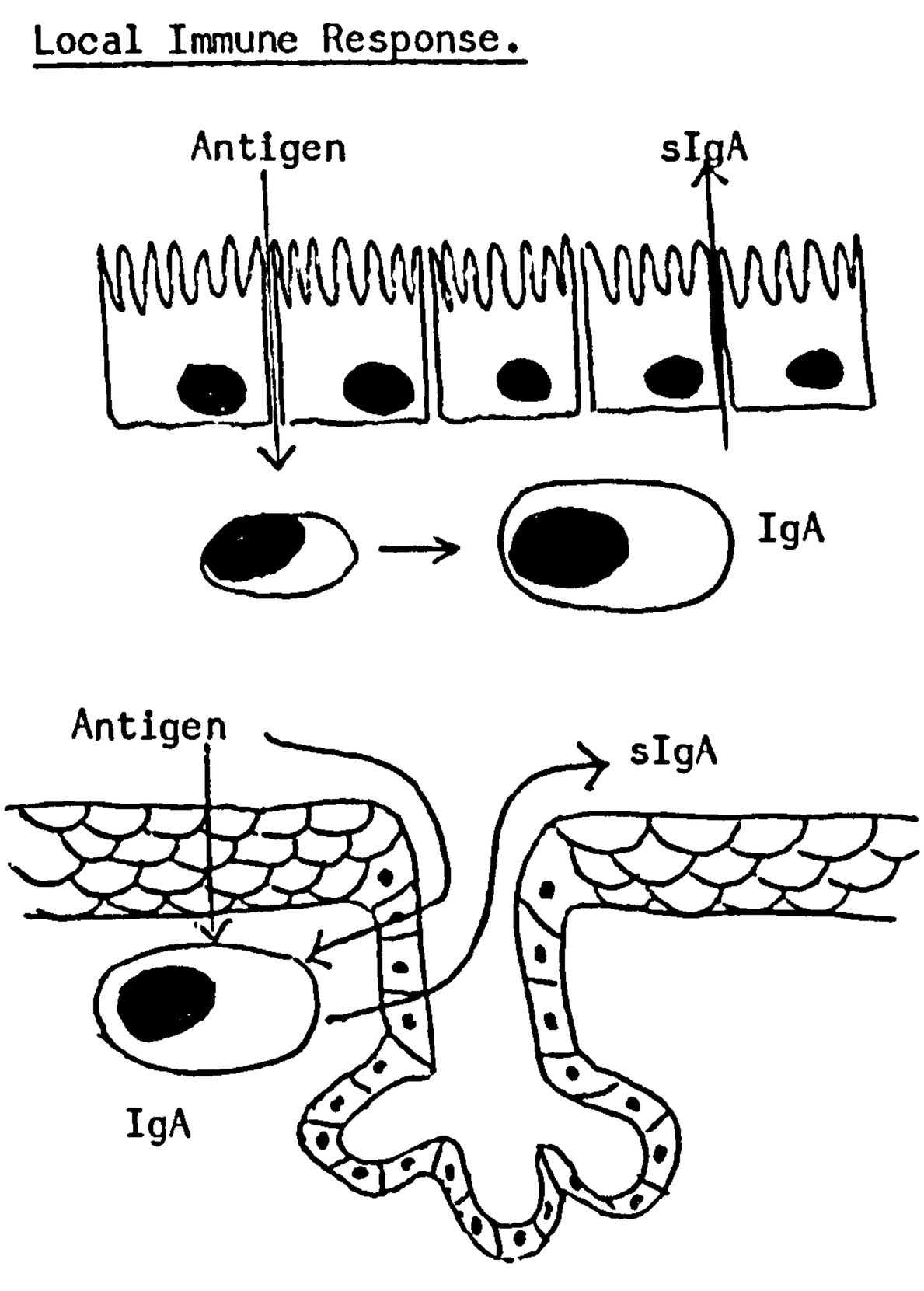


Figure 2. Pathway for Stimulation of the sIgA Associated Immune Response.

A. Local sIgA response restricted to the site of antigen stimulation. (Mestecky et al. (1978 b)).

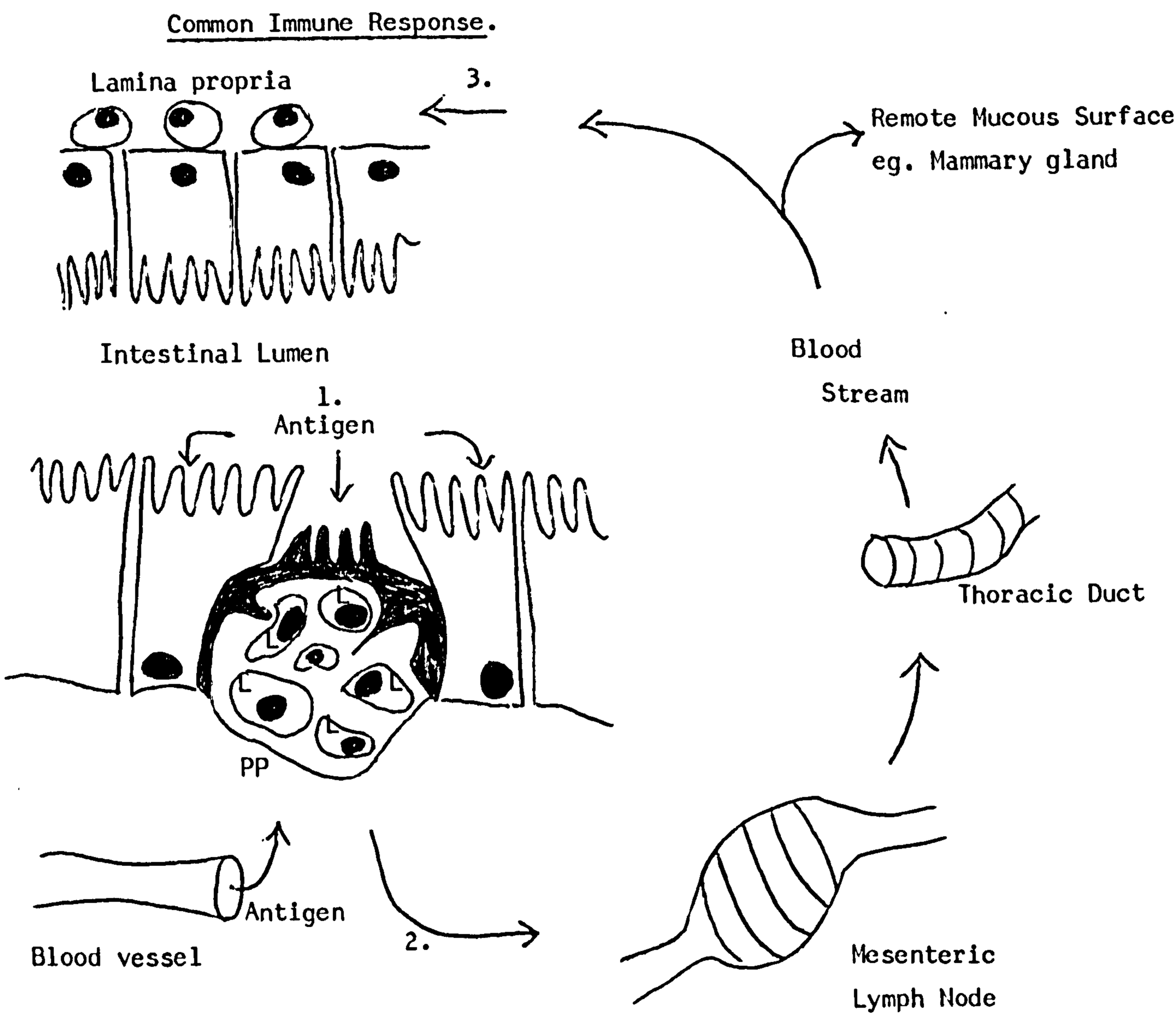


Figure 2. (continued).

B. Lymphocytes (L) within gut-associated lymphoid tissues (GALT) primarily Peyer's Patches (PP) of the ileum are stimulated by antigens entering from the intestinal lumen:

1.via specialised epithelium (M cells) across conventional absorptive cells or from the systemic circulation. Lymphoblasts migrate to mesenteric nodes for further maturation and

2.enter the systemic circulation as plasmablasts to redistribute along intestinal mucous surfaces and

3.produce sIgA antibodies in response to absorbed antigens.

(Walker and Isselbacher ,(1977))

1.4 PRODUCTION AND TRANSPORT OF SECRETORY IgA

Irrespective of origin, the IgA producing cells, once situated at the sub-mucosal surfaces become sites of secretory IgA synthesis. Local synthesis has been confirmed both by in-vitro organ culture and by Fluorescent antibody techniques. Secretory component synthesised in the epithelial cells appears to function on the membrane of the epithelial cells as a receptor for IgA dimers bound by J-chain; the subsequent assembly occurs inside the cell during the selective transport of IgA to the mucous surface. (Brandtzaeg (1978)). The completed secretory immunoglobulin molecules are released from the epithelial cells by reverse pinocytosis.

In the absence of IgA, for example in selective serum IgA deficiency, there is a compensatory increase in the secretion of another polymeric immunoglobulin - namely IgM. This antibody is synthesised by the same mechanism as sIgA. Polymeric immunoglobulins, in association with S.C. appear to represent an adaptation of immunoglobulins for optimum function and survival in secretions.

The role of the secretory component in transport is unclear. (Lamm (1976)). It has been suggested that this glycoprotein represents a specific receptor site for polymeric immunoglobulins which is essential for it's uptake and transport through epithelial cells. An alternative role may involve protection of sIgA against proteolysis. Linkage of S.C. to the IgA dimer protects it both against breakdown by lysosomal enzymes during passage through the epithelial cells and also against other enzymes present on the mucous surface. It is possible that accumulation of IgA plasmacytes at the site of sIgA production is the consequence of a selective homing process mediated by the presence of S.C. on the surface of exocrine epithelial cells. (Brandtzaeg (1973)).

Regulation of B-cell antibody synthesis appears to be mediated by T-cells and macrophages as well as by humoral substances derived from such cells. The regulatory mechanisms for the production of IgG and IgM are well documented. Less is known, however, about mechanisms for sIgA production. Elson et al (1978) have shown that mitogenic stimulation of T-cells with Concavellin A markedly suppressed IgM biosynthesis in all tissues studied but the effect of IgA biosynthesis varied according to the tissue examined - for example in the spleen IgA biosynthesis was suppressed whereas in Peyer's Patches production was enhanced. This evidence suggests a separate T-cell regulatory system for sIgA production.

1.5 PROPERTIES OF SECRETORY IgA

In describing the properties of sIgA it should be noted that they begin at birth. The essentially sterile gastrointestinal tract will, within several hours of birth, be colonised by many species and strains of bacteria. The factors influencing the resulting 'normal' flora are many but could be due in part to the presence of sIgA ingested in colostrum. This regulatory function of sIgA may also be reflected in the colonisation of respiratory and genitourinary tracts.

Colostrum is a rich source of antibodies; many of the secretory type. These antibodies, together with maternal IgG, which has crossed the placenta, play a role in the development of resistance to infection by the infant. Some animals, for example bovines, are born agammaglobulinaemic because maternal IgG does not cross the placenta. If colostrum is with-held many of the calves will succumb to infection within a short period of time. Calves therefore receive all of their immunologic defences post partum.

In the human, the situation is more complex, not only because gammaglobulins cross the placenta, but because there is little evidence of significant adsorption of antibodies from the gastro-intestinal tract (G-I tract) post partum. Therefore, the role of colostral antibodies must be at the local gastrointestinal mucosal level.

G-I tract permeability following birth is a species characteristic. In humans the G-I tract is impermeable to intact proteins; in other animals there is a transient period of permeability ranging for 2 - 3 days in cows and sheep to 15 – 20 days in the rat.

The human neonate, at birth, possesses no detectable IgA either in serum or secretions. In saliva, however, there is free secretory component. The infant, therefore, appears to be prepared to develop a competent secretory immunologic system and this further supports the observation by Tomasi T. B.(1974) that the sIgA system develops earlier than the serum IgA system.

As yet, it has not been established whether IgA antibodies can mediate any secondary effect or functions following interaction with antigen or whether differences exist in this aspect between IgA subclasses. Difficulties are encountered in obtaining preparations of sIgA free from contaminating antibodies of other classes and several discrepancies have been reported in connection with the properties of IgA antibodies. It is unclear whether IgA antibodies can fix complement Götze and Muller-Eberhard (1971) have shown that although IgA immunoglobulins cannot fix complement by the classical pathway, serum type IgA 1 and IgA 2 and secretory IgA proteins are effective activators of the alternate pathway if artificially aggregated. However, in one series of experiments by Colten and Bienenstock (1974) the interaction of sIgA with antigen on the surface of red blood cells failed to activate the alternate pathway.

In addition, it is not certain whether all the necessary complement components are present in external secretions or, if so, that they can function in such an environment. (Ballow (1974)). Secretory IgA antibodies have been reported to interact with complement and lysozyme together so as to bring about bacteriolysis (Hill and Porter (1974)).

Opsonisation is a phenomenon that can either be mediated directly by the Fc portion of an antibody or indirectly via complement. In the case of IgA antibodies, the ability to opsonise is controversial. (Kaplan (1972)). More recently Reynolds et al. (1978) have shown that IgG is the most important opsonic antibody in the lower respiratory tract and that sIgA is relatively ineffective as an opsonin.

The ability of sIgA to counteract infectious micro organisms is well established. The following section will demonstrate however that this is probably not its sole protective function. Under natural conditions, especially in the G-I tract, IgA antibodies are undoubtedly being formed against a great many environmental and ingested antigens. This problem is demonstrated by observations that IgA deficient persons tend to have increased levels and incidence of humoral antibodies directed toward antigens derived from food and intestinal organisms. (Buckley & Dees (1969)). It is presumed that these antibodies are formed because the defective IgA system allows molecules which would normally be prevented from absorbing, to penetrate the intestinal lining. It has been further postulated that the absorption of excess foreign substances is a factor in the high incidence of autoimmune phenomena in these individuals. (Lamm. (1976)). It may be that the ability of sIgA to combine with antigens and to prevent absorption may explain the predominance of the IgA class in external secretions.

Although the inflammatory reaction is thought to play a protective and therefore a beneficial role, it is often an inappropriate and poorly controlled mechanism. Many of the consequences of inflammation are deleterious to the host. For example, in the case of allergic inflammation, it would seem that the body would be better off if such an immune response did not occur. (Thomas (1971) . and Lamm and Stetson (1972)). When immune reactions are mediated by antibody, the inflammatory consequences are due in great measure to the "effect functions" of the Fc regions. (e.g. complement fixation, anaphylaxis, chemotaxis etc). The α- chain constant regions do not mediate an effect or function of major significance in vivo. This lack may be an evolutionary process selected to avoid the undesirable consequences of antigen-antibody reactions. IgA blocking antibodies have a protective role in that they complete for antigens with reagins of the IgE class (Turk et al. (1970) and Stokes et al.(1974)). The latter report suggests that in Atopic allergy there is an overstimulation of the IgE system because of a qualitative defect in IgA production.

1.6 RECOVERY FROM AND RESISTANCE TO VIRAL INFECTIONS

For a clearer understanding of the importance and functions of secretory immunoglobulins it would be appropriate to consider their phylogenetic place amongst mechanisms protective against viral infections.

Recovery from and resistance to viral infection are determined by the interaction of specific and non-specific host factors. Their functions are to prevent establishment of infection and if infection has already started, to interfere with virus replication to limit the spread of infection and finally to eliminate the virus. To acheive this a complex host defence mechanism is required.

In primary infection of the respiratory tract, non-specific host resistance factors represent the main line of defence during the first few days of infection. These consist of the physical barriers to virus penetration, such as intact epithelium and the integrity of the mucous layer, the upwards current of mucus caused by ciliated epithelium together with phagocytosis by wandering mucophages. The latter factor may not be effective against Influenza and other respiratory viruses which infect only the lining epithelial cells and have no subepithelial invasion but is almost certainly vital in terms of priming the lymphocytes. Another series of non specific antiviral activities is introduced soon after the initiation of infection which in the case of myxo and paramyxoviruses involves lysis of mucus by virus-produced neuraminidase. These are local interferon production, elevated body temperature possibly related to Interferon production and acid pH formation at the inflammatory site. Thus by interfering with the early stage of virus attack, the non specific host defences may be important in determining the outcome of a viral infection.

Final recovery from a fully established infection, however, is influenced by the specific immune responses and although active from an early stage can only be measured some days after the initiation of infection.

The specific host immune responses are probably the main mechanism of host defence against reinfection with the same or antigenically related organisms. These are the cell-mediated immune response mounted by cells of the lymphoid system and the humoral immune response expressed as circulating antibody. Cell-mediated immunity is mediated by sensitised T-lymphocytes and antibodies, which neutralise extracellular virus, are produced by B-lymphocytes. Strong interactions occur between the B and T-cell-mediated immune responses to viral infections and other factors such as complement, polymorphonuclear leukocytes and other secondary effectors.

Secretory immunity refers to both antibody and cell-mediated reactions which occur at mucosal surfaces. Systemic antibody and systemic cell-mediated immunity have long been accepted entities, whereas local antibody, primarily secretory IgA has been vigorously studied only in the last decade or two. (Tomasi (1972)).

Much of the research concerned with examining the production of secretory antibodies following virus infection has been carried out during vaccination programmes. Inactivated Influenza virus vaccine is, at present, the only respiratory virus vaccine available. However, despite the fact that vaccines have been available for more than thirty years Influenza remains the only major epidemic disease which has not been brought under control. A thorough investigation of the different antibody responses is essential before the efficacy of these vaccines can be evaluated.

In Poliomyelitis the role of secretory antibodies has been made clear by the work of Ogra and Karzon (1971). Their findings can be summarised as follows: circulating IgG type poliomyelitis antibodies
a) protect against the paralytic symptoms of the infection and b) have little or no influence on the multiplication of the virus in the mucosal cells of the digestive tract, its subsequent isolation from the infected individual or its circulation throughout the community.
By contrast, secretory antibodies
a) prevent the development of paralytic symptoms by neutralising the virus at the portal of entry and b) prevent the virus from multiplying in the mucosal cells of the intestine; stop its excretion from the infected individual and also stop its subsequent spread in the community. For these reasons as far as Poliomyelitis is concerned circulating IgG antibodies may be thought of as the primary factor in host immunity whereas secretory antibodies are the primary factor in collective immunity. These observations will then be useful in forming an understanding of the role of secretory antibodies in protection against other viruses and in particular Influenza.

Two main criteria are generally used to determine the importance of antibodies in protection against infectious diseases. The first is the correlation between antibody level and susceptibility to infection. The other relates to the possibility of preventing its symptoms by administering antibodies.

The role of serum antibodies in protecting against Influenza has not been defined completely. Francis in 1937 was the first to show that circulating antibody levels correlated with immunity and several epidemiological surveys between 1960 - 70 have shown that a fall in 'herd' serum antibody levels to influenza virus variants is considered to be the main reason for the occurrence of epidemics. (Gordon and Belova (1973); Foy et al.(1973)and Stiver et al.(1973)).

However, several studies have shown significant numbers of people with high titres of antibody becoming ill and vice versa. (Davenport (1961) and Morris et al, (1966)).

Recently Butler (cited in Kilbourne et al. (1973)) has suggested that influenza immunity as assessed by a H-I serum antibody titre of 1:32 and higher does not necessarily indicate that these antibodies are able to protect against infection. The antibody level may only reflect the person's general immune status.

The role of secretory antibodies in protecting seemed to have been established by Francis in 1943. However, the research necessary for optimising the application of inactivated influenza virus vaccines is an ongoing problem. More recent confirmation of anti-influenza immunity in ferrets was carried out by Shore et al. (1972). The resistance of these animals to reinfection gave a positive correlation with levels of secretory antibody; titres of circulating antibodies did not correlate.

Several investigators have observed that local application of inactivated influenza vaccine stimulates the production of neutralising IgA antibodies in nasal secretions and induces protective immunity. (Waldman et al. (1968, 1969 and 1970);Kasel et al. (1969);Waldman and Coggins (1972); Liem et al.(1973 a);André et al.(1976)). Studies by Perkins et al. (1969) have shown that local immunisation against rhinovirus is important and that significant protection against viral challenge followed in those vaccinees who developed secretory IgA nasal antibodies. Both parenteral and aerosol vaccine groups had serum antibody titres after immunisation.

However in other studies (Tyrrell et al.(1970); Edmondson et al.(1971);Stuart-Harris (1973))protection conferred after vaccination by the intranasal route was found to be poor; possibly due to differences in the method used for the administration of vaccine. (Liem et al.(1973 b)).

Parenteral administration of inactivated influenza vaccine provokes a secretory immune response simultaneously with the serum response under certain circumstances. In 1973,Dowdle showed that if the concentration of antigen in high, secretory and circulating antibodies will be detected. The strain of virus used, recipient's age and previous antigenic experience are also important. Children below the age of 2 years respond poorly even when high doses of inactivated virus antigen are used.

Passive immunity can be induced rapidly by the intramuscular or intravenous administration of antibody formed in other individuals. However, this immunity tends to be short-lived. The parenteral administration of antibodies isolated from the sera of donors immunised with Influenza vaccine has been used widely in the treatment of patients , especially small children with 'hypertoxic' forms of Influenza, If these antibodies are administered in the early stages of the disease (days 1 and 2) they prevent toxicity, shorten the duration of fever and prevent the development of pneumonia.

Nechaev et al. (1938) were the first to administer hyperimmune serum intranasally for medical purposes. This route of administration, like the parenteral route is effective in terms of reduced numbers of cases, fewer complications when disease occurs and a shortened duration of disease.

The available literature concerning Influenza vaccination is vast and confusing. Four types of Influenza vaccine are currently available. They are attenuated or inactivated and are administered parenterally, intranasally or both. In addition adjuvants can be used. In the numerous studies, researchers have examined serum and secretory responses. Problems have arisen in the timing of the trials (i.e. problems with outbreaks of wild type influenza) and in the methodology of secretion collection and testing for antibody content. It is virtually impossible to draw comparisons between trials due to the high number of variables.

What is clear, however, is that since secretory immune responses are important in protection against respiratory viral infections, a knowledge of the optimal methods of stimulating such antibodies on mucosal surfaces is essential in determining the best procedures for immunisation.

Recent data suggests that the secretory component of sIgA may itself play an important role in protection. An example was reported recently by Strober et al. (1976) They recorded the case of a 15 year old boy with chronic intestinal Candidiasis who had normal serum IgA levels but who lacked IgA in his secretions. He appeared to be deficient in secretory component. It is thought that secretory component may either provide a homing signal for IgA cells or may provide a stimulus for differentiation or proliferation of IgA precursor cells which move into mucosal areas. An SC deficiency in mucosal cells may therefore induce defective homing of IgA producing cells, resulting in the lack of secretory IgA synthesis and lack of protection against local infection which was observed in this patient.

1.7 QUANTITATION OF SECRETORY IgA IN SECRETIONS

In contrast to the measurement of serum IgA by standard serological methods, the quantitation of secretory IgA in biological secretions is an area of complexity and uncertainty. Some of the major difficulties are summarised below.

a. Expressing the concentration of secretory IgA

The concentration of IgA in secretions can be expressed as a percentage of total protein or, alternatively, as the IgA to albumin ratio. This latter method is convenient when the sample differs from the original fluid by an unknown factor, for instance, with nasal fluid that has to be collected by washing the nostrils with a standardised amount (eg. 10 ml) of an isotonic solution.

Unfortunately there is no satisfactory technique for measuring total protein concentrations in complex mixtures such as saliva. Inaccuracies occur through the use of single standards such as albumin or γ-globulin which give results which are,at best, r elative. When colorimetric reactions (Folin or Biuret) are used technical difficulties may result in the colour development (Kabat & Mayer,(1967)). In practice this means that none of the available methods provide absolute values for the total protein content of a secretion (Tomasi (1972)).

An additional complication exists in the fact that for many secretions the total protein concentration varies widely within the same individual and depends upon the rate of fluid secretion. In addition to measuring the total protein content of a secretion, it is desirable to determine the volume of secretion collected over a given time period. The immunoglobulin concentration can be expressed, as with urine, in milligrams excreted per 1000ml or per 24 hours.

As expected, where flow rate has been measured, it has been found that the IgA concentration of some secretions drops significantly with increasing rates of secretion. Brandtzaeg (1970) showed that the IgA concentration of parotid saliva fell 3 - 4 fold while the rate of secretion (ug/minute/gland) was 3 times increased after stimulation. Following prolonged continuous secretory stimulation IgA secretion rates remain essentially unchanged.

Another aspect of this problem is that there is a large variation in the monomer to polymer ratio of IgA in various secretions. This ratio ranges from 1:20 to 1:1 (Brandtzaeg,(1970)). The molecular weight of precipitating antigen influences the result of measurement techniques in gel (such as single radial immunodiffusion) and marked deviations may be observed in the determination of IgA as a consequence.

In view of these problems quantitative levels of IgA in secretions should therefore be considered as a mere qualitative indication of its presence (Samson,(1973)).

b. Collection of specimens

Two methods have achieved common usage to collect respiratory tract secretions; squeezing the adsorbed secretion from cotton wool tamons (Burnet et al. (1939); Francis et al. (1943); McKerscher,(1972) and Smith,(1975)) and washing the mucous surface with an isotonic solution. (Remington et al. (1964) and Shvartsman and Zykov,(1976)). Both methods were used in this project and are outlined in detail in Chapter 2. (2.1.1 and 2.2.1)

Tears may be collected on standard sized hard filter paper strips held in the corner of the eye or into standard sized polypropylene sponges which when sufficiently wetted are transferred to small vials containing a measured amount (0.1 - 0.2 ml) of isotonic solution. A rough quantitation of tears collected can be achieved by these methods. The volume of fluid collected can be estimated either from the length of paper which has been wetted or from the contents of the sponge. Tears have also been collected by using cotton tipped applicator sticks of uniform dimensions held in the inner canthus of the eye after tears have been stimulated with vapour from freshly peeled onions.

Saliva from the parotid gland may be collected using one of a number of different devices which have in common a cap which is fitted with two concentric rings to cover Stensen's duct. The outer ring communicates by a tube to a device for applying mild suction in order to hold it in position over the orifice of Stensen's duct and to prevent contamination of the parotid saliva with other oral secretions. The inner ring is the collection chamber which allows parotid secretions to flow via a tube to a collecting vessel. (Shannon and Chauncey (1967)).

c. Losses in storage and concentration

During storage losses of sIgA of the order of 10 - 20% may occur at -70^{o}C after a few months. Free secretory component concentrations appear to be less affected by storage (Brandtzaeg and Baklien (1976)).

Freezing at -70^{o}C and thawing once or twice seems to have little discernible effect on the titres of antibody activity measurable in nasal secretions and, since, these secretions are often contaminated with bacteria prompt freezing is a most useful way to store secretions until they can be studied. However hyaluronic acid derivatives become insoluble as a result of one freeze-thaw cycle and freezing is to be avoided if these substances are to be studied (Rossen et al. (1971)).

Since many external secretions contain concentrations of immunoglobulins (particularly IgG or IgM) too low to measure by most of the commonly used techniques, it is necessary to concentrate fluids. Lyophilisation has been used extensively. The dried fluids are reconstituted up to one tenth of the initial volume or up to the concentration corresponding to 20 mg. of IgA in 100 ml.

The use of lyophilisation in practice is questionable. According to several authors cited in Rossen, (1971) from 30 - 70% of IgA is denatured by lyophilisation.

It has proved generally more suitable to concentrate secretions using some means of withdrawing water and electrolytes through a semipermeable membrane. A variety of hyogroscopic agents are also available, for example, Sephadex G-200 (Pharmacia) and Lyphogel (Fisher Scientific Co.), for removal of water for concentration. These have the disadvantage of high cost and the possibility that 'something else' may be removed.

Despite the range of available methods for the primary treatment of secretions most investigators have limited this to homogenising and removal of insoluble particles by centrifugation.

d. Methods of secretory IgA assay

The methods available for the detection and assay of IgG antibody to viral antigens are of two types, those which are multicomponent systems and those which are based on two component systems. Amongst the multicomponent methods are several which have been used routinely for many years, including virus neutralisation tests, haemagglutination-inhibition tests (H.I), complement fixation (C.F), Immunofluorescence (I.F) Radioimmunoassay (R.I.A.) and more recently enzyme linked immunosorbent assays (E.L.I.S.A.) Essentially these systems depend on testing serial dilutions of test sera with known doses of virus or viral antigen and employing an appropriate indicator system to measure the degree of reaction of antigen with specific antibody.

Several two component systems have been accepted for routine use. These are immunodouble diffusion (I.D.D.) single radial diffusion (S.R.D.) and immunoelectrophoresis (I.E.) techniques. These techniques depend on antigen-antibody reactions in gel.

The quantitation of secretory IgA in nasal secretions can be carried out using techniques which are basically the same as those outlined above for sera. The possible techniques will be described with particular reference to the problems arising when secretory IgA is under test.

i) Neutralisation tests:

These are the most sensitive of the 'classical' immunological reactions. Infectious virus when combined with specific antibody is unable to be taken in by susceptible cells and is said to be 'neutralised'. In principle virus and secretion are mixed, allowed to react for an appropriate time and the mixture inoculated into a host system which can then be examined for signs of infection. Tests are usually carried out in animals, embryonated eggs or monolayers of cells. The advantage of sensitivity is set against the disadvantage that these tests are tedious and time consuming to perform.

ii) Haemagglutination Inhibition Tests : (H.I.)

H.I is a technique used either to diagnose infection or to indicate the immune status of an individual. It is also used to identify the haemagglutinin subtype of influenza viruses.

Inhibition of haemagglutination results when antibody attaches to the virus haemagglutinin. This either blocks red blood cell reactive sites on the virion or eliminates the virus from competition for red blood cells by forming virus antibody complexes.

Although H.I is a simple, highly reproducible test suitable for mass investigation it has a major disadvantage Haemagglutination of viruses may be inhibited by normal components of sera from a wide variety of animals. These non-specific inhibitors can lead to false positive H.I tests. They are usually glycoproteins and comprise of three groups, alpha, beta and gamma, according to virus types and subtypes affected.

Receptor-destroying enzyme (R.D.E.) of Vibrio cholerae and potassium periodate are most commonly used for destroying non-specific inhibitors.

In the use of the H.I test for determining antibody concentrations in nasal secretions, there are problems that have not yet been solved. Whether non specific inhibitors are present remains controversial as does the treatment of specimens prior to testing (Schwartsman(1976)).

iii) Radioimmunoassay (R.I.A.)

R.I.A. has become a highly specialised technique. The basis of the assay is that the concentration of an unknown antigen is determined by measuring its ability to compete with a fixed amount of radiolabelled antigen for a limiting amount of antibody. Standard curves of this inhibition are produced using known amounts of unlabelled antigen. Total amounts of IgA in secretions can be measured using this technique. R.I.A. is highly sensitive. However complex equipment is necessary and because of the medical hazards posed by the use of isotope labels, highly trained personnel are required.

iv) Immunofluorescence (I.F.)

Fluorescent dyes such as fluorescein and rhodamine can be coupled to antibodies without destroying their specificity In 1941 Coons showed that such conjugates would combine with antigen present in a tissue section and that the bound dye could be visualised in the ultra-violet microscope, using appropriate filters, through the emission of fluorescence. In this way the distribution of antigen throughout a tissue and within cells can be demonstrated. The method can also be used for the detection of antibodies directed against antigens already known to be present in a given tissue section or cell preparation. The end-point being that dilution of antiserum showing a 50% reduction in brightness. This rapid, elegant technique has the slight disadvantage of depending on the subjective visual assessment of fluorescence unless a photometer is available. This instrument is beyond the financial capabilities of most laboratories.

If this technique is used to measure secretory IgA against a specific viral antigen there are slight technical problems due to the non availability of appropriate commercial antisera and conjugates. In addition nasal washings tend to be heavily mucoid. Mucus gives a high undesirable background fluorescence and introduces the possibility of 'missing' low antibody levels.

v) Enzyme-linked Immunosorbent Assay (E.L.I.S.A.)

Serological methods are playing an increasingly important role both in the diagnosis and in the epidemiological study of disease. Simple, inexpensive methods for large-scale application are urgently needed. The enzyme immunoassay methods were developed by Engvall and Perlman (1971 and 1972). The methods are based on the use of antibodies or antigens which are linked to an insoluble carrier surface.

This is then used as a base for the binding of the relevant antigen or antibody from the test solution. The complex is then detected by means of an enzyme-labelled antibody or antigen. The ensuing degradation of the enzyme substrate, measured photometrically, is proportional to the concentration of the unknown 'antibody' or 'antigen' in the test solution. Under laboratory conditions these methods can be as sensitive as R.I.A., but can also be adapted as simple field-screening procedures.

Åkerlund (1977) used the technique to quantitate secretory IgA in biological secretions. Despite rapid advances there are still several problem areas. Firstly the purity of the antigen would appear to be critical and secondly the guide lines for defining an end-point titre are very vague.

In the future, ELISA systems will surely be used routinely as a supplement to virus isolation and immunofluorescence techniques to detect viral antigens in clinical specimens. (Bishai et al.(1981)). A variation of the method allows ELISA to detect specific secretory IgA in human sera as a diagnostic test for viruses. Rotavirus antibody can also be detected in this way. (Grauballe et al.(1981)).

Precepitation Reactions in Gels

i) Immuno Double Diffusion (I.D.D.)

When an antigen solution is mixed in 'optimal' proportions with homologous antiserum, a precipitate is formed. The precipitation reaction can be visualised in gels. In the double diffusion method of Ouchterlony (1970), antigen and antibody placed in wells cut in an agar gel, diffuse towards each other and precipitate to from an opaque line in the region where they meet in optimal proportions. A preparation containing several antigens will give rise to multiple lines.

I.D.D. techniques are of considerable value as qualitative tests for the identification of antibodies in test sera but are not readily adaptable to quantitative studies.

ii) Single Radial Immunodiffusion (S.R.D.)

The single radial immunodiffusion test provides a simple assay system for antibodies or antigens based on the principles first described by Mancini in 1965. For antibody measurement antigen is incorporated uniformly throughout a matrix (usually a gel) and antibody is added to a well cut in the gel. The antibody diffuses rapidly and at optimum proportions of antigen/antibody a visible zone of precipitate forms. The size of the zone is proportional to the concentration of antibody applied to the gel in a given time period. Therefore using serum dilutions of known concentration a standard graph of zone size against antibody concentration can be produced and used to determine antibody concentration of unknown specimens.

This method is commonly used for the determination of secretory IgA in concentrated fluids.

iii) Single Radial Haemolysis (S.R.H.)

A technique which has recently been accepted in the diagnostic laboratory is single-radial haemolysis. The method utilises the ability of specific antibodies in the presence of complement to lyse eythrocytes which have been sensitised by treatment with certain viral antigens eg. Rubella, Influenza. When performed in agarose gels the technique enables antibody to be quantitated by measurement of the size of zones of haemolysis surrounding wells in the gel to which antisera have been added.

The value of the S.R.H. technique for the measurement of secretory IgA has not been documented but Schild et al. (1975). indicated that its sensitivity in detecting serum antibody to haemagglutinin is equivalent to or greater than conventional H.I tests.

The method is simple, rapid and does not require pre-treatment of sera since it is not affected by non-specific inhibitors in animal sera. This technique is suitable for large scale serological surveillance for antibody to the haemagglutinin antigen such as is required when a new influenza variant emerges.

iv) Immunoelectrophoresis

Immunoelectrophoresis has proved to be a valuable tool in the qualitative investigation of proteins, particularly human serum immunoglobulins. Classically, the technique involves the separation of proteins held in a gel support medium by passing an electric field across the gel. Visualisation of the separated proteins is achieved by using specific antisera. Initial separation of the proteins depends on the charge carried by each protein. Polyclonal immunoglobulins being of varying amino-acid composition will exhibit a spectrum of mobility moving as a broad band on electrophoresis and producing a pattern of smooth arcs with monospecific antisera to IgG, IgA and IgM.

Further developments of the principle have combined electrophoresis with immunoprecipitation in which movement in an electric field drives the antigen directly into contact with antibody. Counter current electrophoresis may be applied to antigens which migrate towards the positive pole in agar. This qualitative technique is considerably more sensitive than double diffusion (Ouchterlony (1967)). Rocket electrophoresis is a quantitative method in which antigen diffuses, under the influence of an electric field, into a layer of agar containing specific antiserum. The antigen-antibody rocket-shaped precipitate length is proportional to the concentration of antigen in the well and to the duration of electrophoresis. The method is more sensitive than simple

radial diffusion and can be used to measure immunoglobulins in small samples of dilute and unconcentrated biological fluids.

Standards

Whatever method of gel-technique adopted it is essential to use an appropriate standard for the immunoglobulin under study because these techniques are very sensitive to differences in molecular size of the antigen. This is particularly important for IgA which occurs in several molecular sizes in secretory fluids. The major species of IgA in most fluids is 11S and so it is best to use an 11S standard isolated from saliva or milk (colostrum) when a technique such as radial diffusion or electro-immunodiffusion is used for quantitation since these depend on size. Using a 7S (serum) IgA standard the concentration of IgA in secretions will be underestimated approximately 3 - 4 fold.

Pooled serum may be used as a working standard but prior calibration is necessary with 11S secretory IgA from colostrum or saliva. (Brandtzaeg et al. (1970) and Tomasi (1972)).

CHAPTER 2

MATERIALS AND METHODS

2.1 VACCINE TRIAL

The present study was carried out in Girvan, South Ayrshire, Scotland. The study was started on 28th November, and completed on 3rd May, the following year. (1977 - 78).

Details about the composition of the vaccine used in the study, the experimental design and the procedure for the study are given in Appendix II.

47 subjects were selected on the basis of low serum H.I antibody titres and 44 of them entered the study. One subject (no. 1719) was withdrawn from the study a few weeks after vaccination (hysterical reaction, admitted to hospital) of the remaining 43 subjects, 22 received Influvac Spray and 21 received placebo under double-blind conditions.

6 weeks after vaccination, blood samples and nasal washings were obtained from all 43 subjects.

4 months after vaccination, 41 subjects were challenged. 2 subjects (one in each group, nos. 1745 and 1732) left the study for private reasons and did not receive the live vaccine. One challenged subject (no. 1729) did not provide a final blood sample (admitted to hospital with myocardial infarction).

2.1.1 COLLECTION OF SPECIMENS

a) Nasal Washings

Nasal washings were collected by the following standard technique. The volunteer was asked to sit with his neck hyperextended and 5ml of phosphate buffered saline (P.B.S.) at pH 7.2 were instilled into each nostril using a 10ml disposable, plastic syringe fitted with a Yale Microlance 21G1½ needle in a protective guard with the tip removed. During this process the volunteer continuously pronounced the letter 'K' to prevent P.B.S. entering the alimentary system. The volunteer then brought his head forward and forcibly expelled the washing into a beaker placed firmly against his top lip. This procedure was repeated until a volume of 5ml of washings was collected. The washings were aspirated with a pipette and centrifuged at 1500g for 15 minutes to remove insoluble material. All nasal washings were tested for the presence of blood contamination using Labstix (Ames Co, Div. of Miles Lab. Ltd) This 'dip and read' test took 30 seconds and registered whether or not blood was present by changing from yellow to blue. The depth of colour was dependent on the amount of blood present. No specimens registered a positive result. The supernatants were stored at-20^{o}C prior to determination of antibody titres by several methods.

b) Blood Samples

8ml of blood were collected into sterile dry containers, allowed to clot, and centrifuged at 2500g for 5 minutes. Separated serum samples were stored in ampoules at -20^{o}C prior to antibody testing.

2.1.2 ANTIBODY MEASUREMENT IN NASAL WASHINGS

a) Total Secretory IgA and IgG

Radioimmunoassay

The Radioimmunoassay(R.I.A.)results presented in this thesis were obtained by courtesy of Dr. D.B.L. McClelland, Director, South East Scotland B.T.S., Royal Infirmary, Edinburgh. Total amounts of secretory IgA and IgG in the nasal washings were estimated using the α-chain and γ-chain assays in routine use at Edinburgh Royal. The basis of this highly specialised technique is that the concentration of an unknown antigen (i.e. secretory IgA) is determined by measuring its ability to compete with a fixed amount of radiolabelled antigen for a limiting amount of antibody. Standard curves of this inhibition are produced using known amounts of unlabelled antigen.

In this particular assay system the antibody was immobilised in an insoluble support (Sepharose 4B particles). This aids the separation of bound antigen from free antigen. The purer the antibody used, the more efficient the system.

It should be noted that the following standards were used:

α-chain assay: Human colostrum secretory IgA

γ-chain assay: Purified IgG from the pooled sera.

(Yap et al. (1979)).

2.1.2

b) Specific Secretory IgA

i) Quantitative Haemadsorption

This technique was used to assay the neutralising antibodies present in unconcentrated nasal washings against Influenza type A/Victoria (H_3N_2). The reasons for choosing this method were specificity and reliability plus the ability to correlate the results with those from previous trials and from two trials running concurrently in Rome and Zagreb.

The technique used was evolved by Dr. N.B. Finter (1967) and was a modification of a previous method of Interferon Assay. (Finter, 1964).

The principles involved in Quantitative Haemadsorption are that red blood cells are added to virus-infected cell cultures, excess red cells are washed away and the haemadsorbed red cells are then lysed by adding distilled water. Each culture thus provides a solution of haemoglobin at a concentration which is proportional to the number of haemadsorbed red cells, and which can be measured with a spectrophotometer. In this way relative amounts of growth of a haemadsorbing virus can be measured precisely. This report describes the use of this quantitative haemadsorption (QH) method in the assay of neutralising antibodies.

Materials

Virus: Influenza type A/Victoria/3/75 (H_3N_2) supplied by W.H.O. Geneva.

Specimens: Nasal Washings from human volunteers (see Protocol, Appendix II)

Tissue Cells: Monolayers of secondary African Green Monkey Kidney (AgMK) Cells (supplied by Flow Laboratories, Irvine) in 4" x 1½" glass tubes plugged with silicone stoppers.

Blood Cells: Human O red blood cells supplied by West of Scotland, B.T.S., Law Hospital.

Assay for Neutralising Antibodies

1. Nasal washings were inactivated at 56°C for 30 minutes
2. Serial doubling dilutions of 0.5ml volumes were made using 199 and Hepes, serum free medium as diluent.
3. To each dilution 0.5ml virus was added at the predetermined concentration (1:100).
4. The virus/antibody mixtures were incubated at room temperature for 90 minutes.
5. From each dilution of virus/antibody mixture, 0.2ml amounts were inoculated into four tubes of AgMK cells.
6. The mixtures were adsorbed at 4°C for 1 hour prior to adding maintenance medium.
7. The cultures were incubated at 37°C for 22 hours, in a stationary position.
8. Following incubation the medium was decanted from each tube and replaced with 2ml of 0.4% human O red blood cells (r.b.c)
9. The tubes were placed at 4°C for 30 minutes to allow haemadsorption to take place.
10. The r.b.c. were in turn decanted and the tubes were washed with chilled P.B.S. to remove excess r.b.c. (twice)

11. The haemadsorbed cells were lysed by adding 4ml distilled water. The tubes were agitated gently and left for 30 minutes at room temperature.
12. The concentrations of haemoglobin in the resulting solutions were measured in an Unican SP800 spectrophotometer at a wavelength of 410nm.

The following controls were included:

i) Virus controls: These were four tubes of AgMK cells which were inoculated with virus and diluent only and represent 100% haemadsorption.

ii) Cell Controls: These were four tubes of cells containing two volumes of diluent and detected any non-specific adsorption of r.b.c.

Selection of the amount of virus for the Quantitative Haemadsorption Assay

1. 4-fold dilutions of 0.5ml volumes were made using 199 and Hepes, serum-free medium as diluent.
2. 0.1ml of each vurus dilution was inoculated into four tubes of AgMK cells (including controls).
3. The virus was adsorbed at $4^{o}C$ for 1 hour prior to adding maintenance medium.
4. The tubes were incubated at $37^{o}C$, stationary for a convenient time (i.e. 22 hours).
5. Haemadsorption was carried out as described above (steps 8 - 12).

6. The resulting optical density readings were plotted on a graph against the log concentration of the virus. For use in the test, the dilution of virus was chosen which gave near maximum readings for QH at the selected time.

The results obtained with Influenza type A/Victoria in AgMK cells are shown in figure 3. A suitable dilution of virus was 1:100. The original dilution chosen was 1:20, however, during preliminary testing of the nasal washings it became apparent that the antibody content of the nasal washings was low and consequently a higher dilution of virus was required to increase the sensitivity of the assay.

Methods used in presenting results

An example assay of neutralising antibody in nasal washing specimens is shown in figure 4. The optical density (O.D.) readings at 410nm have been plotted against the corresponding log 10 serum dilutions.

There were two sets of controls in each assay.

1. Uninfected: To detect non-specifically bound red blood cells. This normally very weak solution of haemoglobin was used to zero the spectrophotometer and from a base-line for measuring the ODs of test samples.

2. Infected: To provide a maximum OD value for a solution of haemoglobin resulting from the lysis of haemadsorbed red blood cells.

The difference between the O.D. readings with these controls represents the excess haemoglobin due to growth of the virus. The scale for expresssing other O.D. readings as a percentage of this difference is shown on the right-hand ordinate in figure 4.

From a number of similar assays it has been found that when the results obtained with a wide range of antibody dilutions were plotted, an S-shaped curve could be fitted to the points. Such a curve had a linear portion over a range of O.D. readings corresponding to approximately 25% to 75% excess haemoglobin. A straight line has correspondingly been fitted to the relevant points in figure 4.

The end-point of neutralising antibody activity was taken as the $\log_{10}$ serum dilution at which there was a 50% reduction in the excess haemoglobin due to virus growth.

The end point from figure 4 is 1.17 log QH 50 units.

Use of a Reference Nasal Washing

On the basis of the following (Finter, 1967)

'Within a single assay, parallel dose-response curves are obtainable for all sera (here nasal washings) tested together. Hence if the dose-response curve was precisely determined for any one nasal washing used as a reference (eg. 1717 (IV)), the relative titre of another nasal washing tested in the same assay could be obtained from the result at any single dilution giving an O.D. reading within the linear 25% to 75% excess haemoglobin range'.

Screening

Dilutions of 1:20 were tested against 1717 IV Reference nasal washing. Within a batch the dilutions can be compared with the reference and depending on the displacement from the reference, can be assigned a titre of log QH 50 unit/ml. The screening was carried out in 4 batches and the reference assayed 4 times. If one assigns an arbitary value of 1.28 log QH 50 units/ml (the value obtained in one of the assays) as the titre of the reference nasal washing then the titres of the reference obtained in other 3 assays can be corrected against this titre and the 3 correction factors added to the nasal washing titres within each batch in an effort to standardise the test. Any specimens which gave either no or questionable results were titrated and reassayed.

The Virus, Influenza type A/Victoria/3/75 (H_3N_2) was used in several of the assays described in this chapter. Stock was prepared as follows:

EGG INOCULATION

This simple technique was used to produce large quantities of Influenza Type A/Victoria/3/75 virus.

Method

Fertilised eggs which had been incubated for 10 - 12 days were 'candled' (ie. examined in a darkened room over a bright lamp) to check that the embryo was alive and to mark the position of the air sac and main blood vessels. The shells were sterilised by swabbing with merthiolate. Using a dental drill fitted with a carborundum disc, a small area of membrane was exposed on the side of the egg in an area free of blood vessels. An incision was made in the shell over the air sac to release any pressure produced by subsequent inoculation. The syringe was filled with virus suspension and 0.1ml was injected into the egg through the exposed membrane and into the allantoic cavity. Both openings were then sealed with tape and the eggs incubated for 48 hours.

Harvesting

Following incubation the eggs were chilled at $4^{o}C$ for one hour to prevent haemorrhage during harvesting.

The shell over the air sac was removed to expose the shell membrane which together with the chorioallantoic membrane was removed using sterile forceps.

The exposed allantoic fluid was removed using a Himmelweit pipette and placed in a sterile universal container. The fluid was clarified by centrifugation. The resulting supernatant was tested for viral activity by a haemagglutination test. Those which showed a good yield (i.e. titre 128) were pooled, ampouled into 1ml amounts and stored in liquid nitrogen (-196^{o}C)

The concentration of virus defined above was used in all the tests described which depend on the use of Influenza virus.

2.1.2 ii) Haemagglutination Inhibition

The Haemagglutination Inhibition Test was used as a simple, rapid technique for the measurement of type specific antibody present in unconcentrated nasal washings.

The principles involved are that a standardised amount of virus is added to dilutions of nasal washings under test and incubated under standard conditions of temperature and time. The erythrocytes of an appropriate animal species are added as an indicator of the amount of haemagglutinin neutralised by antibody.

Antigen Preparation - Influenza type A/3/75 grown in allantoic fluids.

Antigen titration:

1. Preparation of 1% human O red blood cell suspension:- Fresh human O.r.b.c. were washed 3 times in PBS by adding buffer and centrifuging for 10 minutes at 2000 r.p.m. The cells were packed by centrifuging in a calibrated tube at 3000 r.p.m. for 15 minutes. The cells were resuspended in buffer to give a 1% suspension.

2. Using a V-well microtitre plate (Dynatech) doubling dilutions of antigen were made in PBS using a microdiluter (0.025ml volumes) and 0.025ml of PBS added to each well.

3. 0.025ml of 1% r.b.c. were added to each well and the plates agitated gently.

4. A control of PBS + 1% r.b.c. should be included.

5. The plates were covered and left at room temperature for two hours.

6. Reading the plates

Positive:	Agglutination i.e. a carpet of cells covering the base of the well.
Negative:	A button of cells on the base of the well.
End point:	Last well to show agglutination. This dilution represents 1 unit of antigen. The antigen should be used at 4 units in the test.

Test System

Using V-well microtitre plates (Dynatech) and volumes of 0.025ml.

1. The nasal washings were heat inactivated at 56^{o}C for 30 minutes and titrated in PBS to give a doubling dilution series ranging from 1/2 to 1/2048.

2. Influenza type A virus suspension was added at a concentration of 4 haemagglutination units.

3. The plates were covered and incubated at room temperature for 1 hour.

4. 1 volume of human O.r.b.c. was added to each well and the plates were incubated for 2 hours at room temperature.

5. The plates were examined and end-points read. The end-point was taken as the last dilution showing significant inhibition of haemagglutination.

Controls

Virus dilutions were prepared in buffer at 4,2,1,½ and 0 Haemagglutination units with buffer replacing serum dilutions. 1 volume of 1% r.b.c. was added and incubated with the test. Readings are graded as 0 for no agglutination (button formation) to 4 for complete agglutination (shield formation) Virus controls should show full agglutination for ≥ 1 HA unit and partial agglutination at ½ HA unit. One well containing 0.025ml buffer in place of virus is included as a cell control.

2.1.3. ANTIBODY MEASUREMENT IN SERUM SPECIMENS

a) Total Serum IgA and IgM

Radial Immunodiffusion

The Radial Immunodiffusion test was used to measure the total concentrations of IgA and M in serum specimens. The plates used in the test were supplied by the Hyland Division of Travenol Laboratories.

Single radial immunodiffusion methods, introduced in 1965 by J.L. Fahey and E.M. McKelvey,are specific for the quantitation of immunoglobulins in biological fluids. The 'precision' method was used, as follows. The wells were filled with a constant volume of serum and zones measured after precipitation was complete (48 hours or longer). The zones were characterized by the presence of a bright ring at the outer edge of the precipition zone.

Reference curves were obtained by plotting the zone diameters squared against the immunoglobulin concentrations contained in the multi-component immunology reference sera. This relationship is generally linear except where zone diameters are small relative to the well diameters. Immunoglobulin concentrations in the test sera are then determined from these reference curves.

Materials

1. Immunoplates (Hyland)

   Set of plates containing antiserum specific for human IgA
   " " " " " human IgM

2. Multi-component Immunology Reference serum. 3 vials, 0.5ml each

3. Precision pipette (3ml) with disposable tips (SMI Micro/Pettor (Hyland)).

Method

1. The immunodiffusion plate was allowed to come to room temperature in its unopened pouch.

2. The plate was removed from the pouch. The cover was removed and the plate allowed to air dry at room temperature until no excess moisture remained in the wells. The cover was replaced firmly until plate was used.

3. The first three wells of one plate were used for the 3 reference sera: the remaining wells on that plate were used for test specimens. Additional plates with the same lot number used in the same run required only one reference serum per plate.

4. With a clean tip attached to the precision pipetter 3µl of the serum were added. Entrapping air bubbles in the pipetter tip must be avoided to ensure that the correct volume is expressed.

5. Serum was added to the well by touching the pipetter tip to the bottom of the well and releasing the serum while slowly raising the tip. Care must be taken not to damage the well during this procedure. The introduction of air bubbles prevents proper diffusion into the gel so they must be avoided.

6. The remaining wells were filled in the same manner.

7. Once the serum had begun to diffuse (ie. when the serum level began to drop) the cover was replaced firmly on the plate.

8. Incubation was at $37^{o}C$ in a moist container for 48 to 52 hours.

9. The diameter of each precipiton zone was measured to the nearest 0.1mm and recorded.

10. A standard curve was drawn and the concentrations of test sera calculated.

2.1.3

b) Specific Serum IgG

Fluorescent Antibody Technique

The Fluorescent Antibody Technique (F.A.T.) was used to measure the specific serum IgG levels against influenza A/ Victoria/3/75 (H_3N_2). It is a simple, sensitive method which enables rapid detection of specific serum antibodies.

A fluorescent dye such as fluorescein is coupled to antibodies without loss of specificity. Such conjugates can combine with viral antigens present in fixed cell preparations and the bound antibody visualised in an Ultra violet microscope through the emission of fluorescence. Using an indirect method the test can be used to measure the concentration of serum antibodies to specific viruses.

Preparation of Infected Cells on Microscope Slides

1. Influenza type A virus was inoculated at a predetermined optional concentration into preformed monolayers of Rhesus monkey kidney cells in glass tubes and incubated for 18 hours. (The concentration of virus and time of incubation were chosen to give a high degree of brightness in 50 per cent of the cells).

2. The medium was discarded and replaced with 1 ml of a Trypsin/Versene mixture containing 0.25 per cent Trypsin (Flow Laboratories) and 0.02 per cent Versene (BDH Chemicals Ltd) in equal proportions.

3. The tubes were incubated at $37^{o}C$ for 5 - 10 minutes until a single cell suspension was formed.

4. The cells were pooled into a conical centrifuge tube, stoppered and centrifuged at 1500g for 5 minutes.

5. The supernatant was discarded into chloros and the deposit resuspended in a small volume of PBS to give an optimal cell suspension of approximately 5×10^{5} cells per ml.

6. The cells were spotted onto precleaned glass microscope slides using glass capillary tubes (Microcaps: Drummond Scientific Corporation).

7. The spots were allowed to air dry and the slides then fixed in acetone for 5 minutes at room temperature.

8. A test slide was stained to ensure that the quality of the batch was acceptable. The rest of the slides were then stored at $-70^{o}C$ until used.

Staining Procedure

1. Microtitre plates, microdiluters and calibrated droppers (0.025 ml volumes) were used to titrate sera in a doubling dilution series from 1/2 to 1/256 in PBS.

2. Slides were retrieved from cold storage and brought to room temperature.

3. The slides were allowed to dry then serum dilutions were overlaid onto the infected cells.

4. Incubation was at 37°C for 30 minutes in a moist chamber.

5. Washing procedure: the slides were rinsed in PBS, washed in PBS for 10 minutes (5 minutes x 2) rinsed in distilled water and air dried.

6. Goat antihuman IgG conjugate. (Kallestad Laboratories) was overlaid and the slides were incubated for a further 30 minutes at 37°C in a moist chamber.

7. The washing procedure as described in 5 was repeated.

8. The slides were examined under a Leitz Ortholux microscope fitted with a mercury vapour illumination source.

When fluorescein is the dye used, positive fluorescence is a bright apple-green colour. In contrast negative specimens have dull green and non-fluorescent staining.

The end point is judged to be the dilution of antibody showing a 50 per cent reduction in the intensity of fluorescence.

c) Specific Serum Haemagglutinating Antibody

Haemagglutination Inhibition Test

The principles and methods are the same as used for measuring specific secretory IgA in nasal washings.

Serum requires pre-treatment however, with an enzyme mixture such as Cholera Filtrate to remove non-specific inhibitors. Two of these enzymes R(eceptor) D(estroying) E(nzyme) and mucinase, eliminate non-specific inhibitors of influenza-virus haemagglutin. This application was first described by Mulder and Van der Veen (1948)

Serum Pre-treatment (Removal of Non-specific Inhibition)

1. A vial of Cholera Filtrate (Philips-Duphar B.V.) was reconstituted by adding 5 ml of distilled water.

2. 5 volumes of reconstituted Cholera filtrate were mixed with 1 volume of serum.

3. Incubation was at $37^{o}C$ for 18 hours to destroy non-specific inhibitors.

4. Before proceeding with the titration the incubation mixture was inactivated at $56^{o}C$ for 60 minutes.

2.2 GENERAL PRACTICE STUDY

The study was carried out in a General Practice on the South side of Glasgow. The study took place during the winter months of 1977 - 78 and 1978 - 79.

146 patients suffering from clinical acute respiratory infections were included in the survey. They ranged in age from 6 months to 80 years and sexes were approximately equally distributed. Each patient was assessed clinically and was re-examined seven days later.

2.2.1 COLLECTION OF SPECIMENS

At each consultation nasal mucus samples were collected by the following standard method. Cotton-tipped swabs were inserted into each nostril as far as possible and left 30 seconds in 'situ'. The swabs acted as irritants and promoted rapid production of nasal mucus. Each swab was rotated, withdrawn and immediately immersed and shaken in 2 ml sterile phosphate buffered saline (PBS) at pH 7.2 and at ambient temperature. Swabs from the nose were also taken for virus and bacterial culture, into appropriate transport medium at $4^{o}C$. In all cases transportation to the laboratory of the collected specimens took place within 2 hours.

The preliminary specimens in this study were nasal washings However, this technique discouraged volunteers from taking part in the study so the above method was preferred.

2.2.2 PROTEIN DETERMINATION

Lowry Protein Estimation

Modification of the method of Lowry et al. (1951) devised to enable evaluation of results in the Multiskan Elisa Reader (Flow Laboratories) and simplify the handling of large numbers of specimens.

Materials

A. 2% Sodium Carbonate and 0.1 N Sodium hydroxide.

B. 2% Sodium tartrate Solution prepared in distilled water.

C. 1% Copper sulphate solution in distilled water

Folin coicalteau reagent.

Method

Prepare solution A — 100 ml of A
1 ml of B
1 ml of C

Solution B. — Folin ciocalteau reagent to 1N (ie. dilute 1/3)

Modification:-

Standard Curve

1. Doubling dilutions of a stock solution of Bovine serum albumin, fraction V (BDH Chemicals) containing 200mg 1dl.
2. Dilutions were made in a flat bottomed microtitre plate (Dynatech M129 A) in volumes of 0.05 ml using a multichannel pipette.
3. 0.25 ml of solution were added and left for exactly 10 minutes.

4. 0.25 ml of solution B were added and left for at least 20 minutes.

5. Samples were read at 619 nm in the Multiskan reader using distilled water as a blank.

6. A standard graph was drawn of protein concentration (mg/dl) against absorbances (absorbance units, AU).

7. 0.05ml of nasal washing were used undiluted in this test and the protein concentration determined by reference to the standard curve.

32 specimens were screened per plate.

2.2.3 SECRETORY IgA DETERMINATION

Rocket Electrophoresis

This rapid, reliable and relatively simple method was used for the determination and quantitation of a single protein (ie. secretory IgA (sIgA))in a mixture. Antigen (sIgA) moves in a layer of buffered agar containing monospecific antibody to sIgA in an appropriate electric field. A stainable precipitate occurs in a 'rocket' shape and the length of the 'rocket' correlates with the amount of antigen (sIgA)present. Under standard conditions of time and electric field strength the method is suitable for measurement of tiny quantities of immunoglobulin in dilute and unconcentrated biological fluids. (Merrill et al (1976)).

Materials

Instrumentation :-

Electrophoresis equipment from Hoechst Pharmaceuticals (Behring Diagnostics).

Reagents:

Antiserum: sheep antiserum secretory IgA plus secretory piece supplied by Dakopatts A/S Denmark.

Agarose: 1% Indubiose in buffer, supplied by l'Industrie Biologique Francaise.

Buffer: Michaelis buffer pH 8.6 (Serva Labs)

Stain: Coomassie Stainer and destainer as described in Dakopatts Manual (Working procedures for DAKO immunoglobulins).

Standards

Low level human IgA reference serum (pooled human serum): Hyland Laboratories.

Secretory Immunoglobulin A (Colostrum): Seward Laboratories.

1. Production of antiserum containing gel plates:

A 1% agarose solution was prepared with barbital buffer, pH 8.6 The specific antiserum was added at a temperature of approximately 55°C. The solution was then uniformly distributed on 10 x 10 cm glass plates on the horizontal table. It is advisable to precoat the plate with a thin coat of agarose to enhance the bonding of gel to plate. Once the gel had set, wells were cut using a gel punch and template (3mm in diameter and 8mm apart).

2. Electrophoresis Conditions:

1. The plates were laid on the carrier plate of the electrophoresis chamber and connecting bridges of surgical lint moistened with buffer were established. The wicks should not overlap the edges of the slide or crooked precipitates may result.

2. The current was switched on and the voltage across the gel adjusted to about 1 volt per cm. 3ul of samples were applied to the wells using a micropipette. The current through the gel should prevent rings of diffusion around the wells.

3. The potential gradient in the gel was adjusted to 7 volts per cm, the lid of the apparatus replaced and the electrophoresis run for 3.5 hours. To keep the temperature in the gel suitably constant the heat produced during electrophoresis must be eliminated. This was achieved by using an inbuilt cooling system. The carrier plate which holds the gels is made of plastic with channels for water cooling. For the time period used, tap water was found to be a suitable coolant with a reasonably constant temperature of $15^{o}C$.

Washing and Staining

1. On completion of electrophoresis, the wells were filled with water and the gel covered with a piece of wet filter paper, taking care to avoid trapping air bubbles. A 2cm thick layer of soft cellulose tissue was placed on the slide and a pressure of about $10g/cm^2$ maintained for 15 minutes. Effective squeezing of the gel was achieved and the liquid-phase of the gel containing non-precipitated proteins was eliminated.

2. The filter paper was peeled off carefully. Any adhering fluff was removed by a light touch and running water.

3. The gel was dried under a current of warm air.

4. The gel was stained by immersion in the coomassie solution for 2 - 3 minutes.

5. The gel was then washed in the destainer until the background was slightly bluish, three changes of destainer were needed. The destaining was done in about 6 minutes.

6. The gel was allowed to dry.

Construction of the Calibration Curve

The rocket heights were measured with an accuracy of 0.5mm. A linear or near-linear curve was obtained by plotting the height of the standard rockets against the amount of antigen applied to the wells. By interpolation on the curve unknown samples were quantitated. I t should be noted that at the onset of measuring sIgA by Rocket electrophoresis the secretory IgA standard (Seward Laboratories) was not in production and the low level serum IgA standard (Hyland Labs) was used. When the secretory standard became available the serum standard was calibrated against the secretory standard and new values assigned to the secretory IgA concentrations. However, for measurement of differences in sIgA concentration it was decided to compare differences in rocket height to minimise discrepancies due to the small rocket height of some of the samples.

2.2.4 BACTERIOLOGICAL AND VIROLOGICAL STUDIES.

The bacteriology results were provided by courtesy of the Bacteriology Department, Belvidere Hospital.

The virology testing was performed by myself and by colleagues in the Virology Department, Belvidere Hospital.

Testing was performed using the standard techniques in current use.

Some text bound close to the spine.

Figure 3. Selection of a suitable dose of Virus for use in Quantitative Haemadsorption Neutralising Antibody Tests.

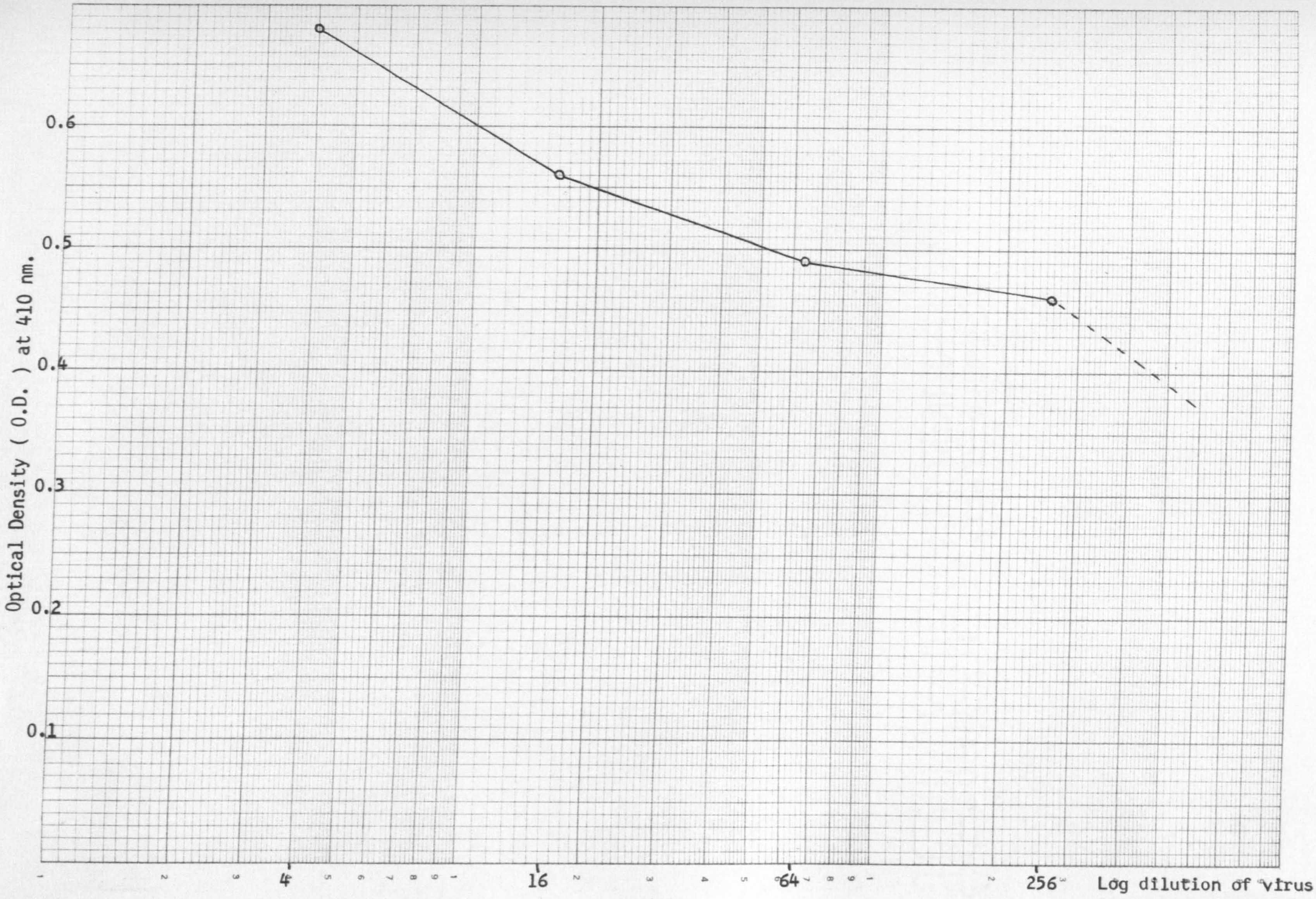
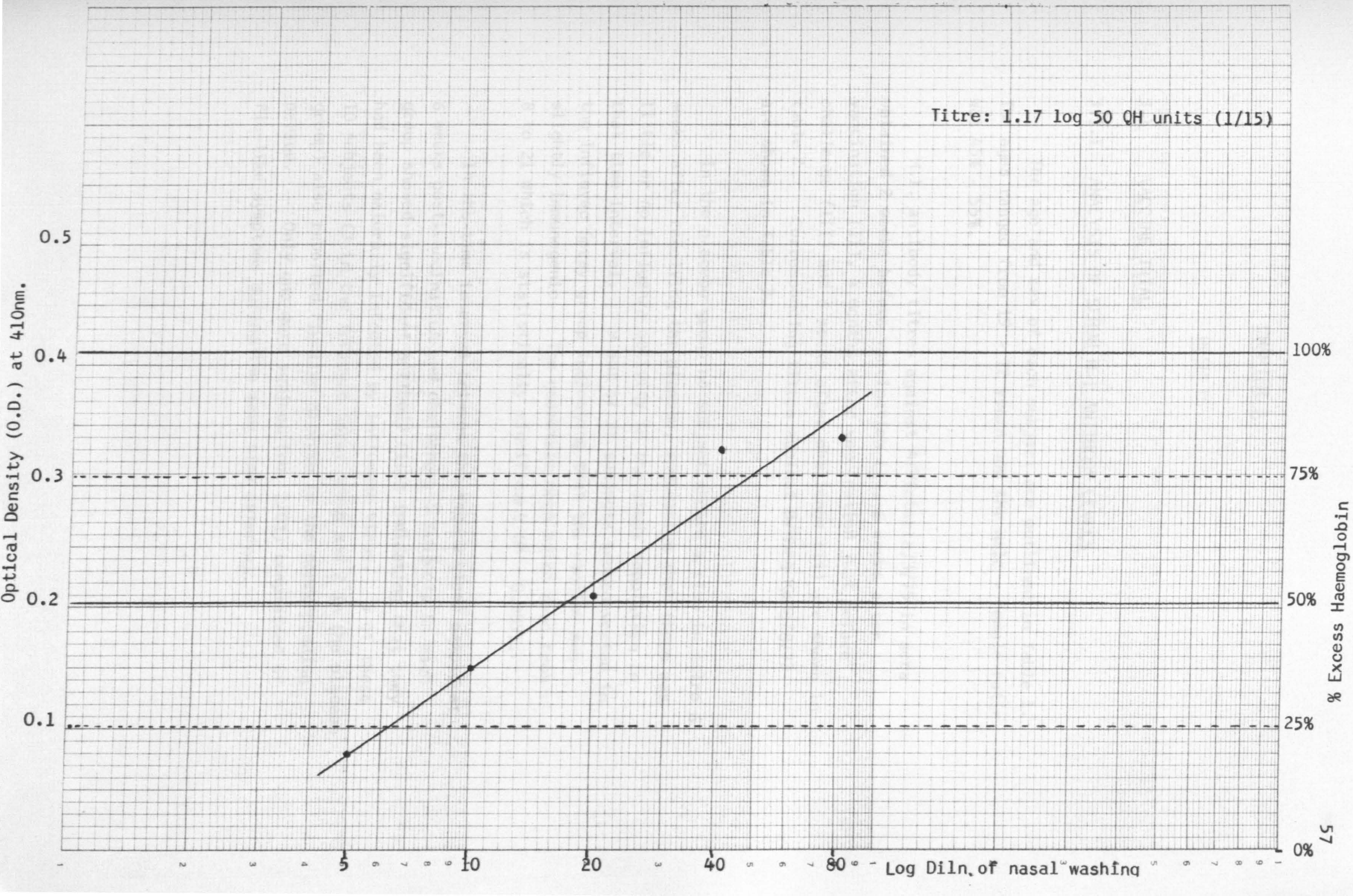


Figure 4. An Example Assay of Neutralising Antibody in Nasal Washing Specimens by Quantitative Haemadsorption.



CHAPTER 3

RESULTS

3.1 VACCINE TRIAL

3.1.1 ANALYSIS OF SERUM H-I ANTIBODY LEVELS

The age and sex of each subject are outlined in Table 1. The ages ranged from 19 - 72 years and the male - female ratio was 45% - 55%.

H.I. antibody titres against A/Victoria/3/75 in sera obtained 2 weeks before vaccination (1), 6 weeks after vaccination (II), 4 months after vaccination just before challenge (III) and 6 weeks after challenge (IV) are shown in table 2. Corresponding titres against B/Hong Kong/8/73 are shown in Table 3.

In the placebo group no subject showed a rise in titre 6 weeks after receiving the placebo, indicating that there was little or no influenza activity in the study population in that time interval. 9 out of 22 subjects seroconverted in the Influvac Spray group indicating that the vaccine was slightly immunogenic. The geometric mean titre rose from 8 to 21 which is statistically significant ($P = 0.005$).

In the time interval between the second blood sample at 6 weeks post-vaccination and challenge, 5 subjects in each group showed significant antibody rises indicating that they had been naturally infected by influenza virus. 5 of these 10 subjects (3 in the Influvac Spray group and 2 in the placebo group) also reported flu-like disease in the corresponding period. Only one more subject (no. 1701) complained of flu-like symptoms during the same time interval.

Thus a small outbreak of influenza occurred during the course of the study. There is no evidence that Influvac Spray gave any protection against natural infection. In fact one subject (no. 1723) who had seroconverted after vaccination was infected. It is also possible that the initial rise in titre was due to natural infection and not the vaccine.

After challenge a further eleven subjects seroconverted (7 in placebo group and 4 in Spray group). If the 10 subjects who were apparently naturally infected are withdrawn from the study (since they would be expected to be immune to a challenge infection) the following protection rate can be calculated for Influvac Spray

$$\text{Protection Rate} = \left(1 - \frac{4/16}{7/14}\right) \times 100 = 50\%$$

With regard to the response after vaccination to B/Hong Kong/8/73, it can be seen in Table 3 that only one subject in the Influvac Spray group produced a significant rise in serum H.I. - antibody titre.
The geometric mean serum H.I.-antibody titre rose in the Influvac Spray group from 50 to 62 after vaccination whereas in the placebo group the corresponding values were 22 and 19.

No adverse reactions related to the vaccine were reported after vaccination. 7 subjects reported common cold (4 in placebo group, 3 in Influvac Spray group) and 1 subject in the Influvac Spray group reported sore throat.

The subjects were originally selected because they had low levels of serum HI-antibodies against Influenza/A/Victoria/3/75. (Titre < 40). No account was taken of initial levels of secretory IgA. Table 4 shows HI-antibody titres of subjects with low initial levels of secretory IgA measured by the Haemagglutination Inhibition test. (Titre < 16). Table 5 is a repeat of table 4 with the exclusion of those subjects who were serologically demonstrated to have contracted wild type influenza virus during the course of the study.

No subject seroconverted in the placebo group whereas 7/11 subjects seroconverted in the Influvac Spray group indicating that the vaccine was more immunogenic in this group. The geometric mean titre rose from 8 to 26. This is statistically significant (P = 0.01/0.005). After challenge a further 8 subjects seroconverted (6 in placebo group and 2 in the spray group).

$$\text{Protection rate} = \left(1 - \frac{2/10}{6/12}\right) \times 100\% = 60\%$$

Table 6 shows the HI-antibody titres when the subjects have moderate to high levels of secretory IgA (titre≥16). Table 7 is a repeat of this group with the influenza sufferers excluded. Although this group is small it can be shown statistically that the Influvac spray does not cause a significant increase in serum antibody levels (P = 0.15). The geometric mean titre rises from 10 to 17.

After challenge there were 3 seroconversions (2 in the Spray group and 1 in the placebo group). Unfortunately there were too few subjects in the placebo group to calculate the protection rate.

Table 1 Age and Sex of each subject

Influvac Spray (n=24)			Placebo (n=23)		
Code number	Sex	Age	Code number	Sex	Age
1701	M	30	1704	M	65
1702	M	54	1708	F	37
1703	M	35	1709	M	42
1705	F	40	1711	F	48
1706	M	45	1713	M	72
1717	F	40	1714	M	72
1710	F	49	1716	F	46
1712	F	45	1717	F	23
1715	M	58	1720	F	27
1718	F	19	1721	F	55
1719	F	57	1722 NE	M	-
1723	F	29	1725	F	34
1724	F	56	1726	F	47
1727	F	38	1728	F	52
1730	F	34	1729	M	44
1734	M	69	1731	M	49
1735	F	32	1732	F	26
1736	F	50	1733	M	55
1737	M	33	1739	F	50
1738	M	54	1740	M	64
1741 NE	M	60	1742	M	39
1743	M	59	1744 NE	M	48
1745	F	25	1746	F	57
1747	F	36			

NE = Subject did not enter study

M = Male

F = Female

Note in Tables 2 through 26

I = pre-vaccination

II = post-vaccination

III = pre-challenge

IV = post-challenge

F = Subject reported flu-like illness in this time interval

* = significant rise

NE = subject did not enter the study

GMT = Geometric mean titre

Table 2 Serum H.I.-antibody titres against A/Victoria/3/75

Influvac Spray (n=22)					Placebo (n=21)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		26	34 F	34	34	1704	34	47	735*	464
1702		8	8	8	8	1708	8	8 F	232*	68
1703		8	8 F	136*	164	1709	8	8	8	41*
1705		8	68*	68	82	1711	8	8	8	26
1706		8	58*	64	64	1713	8	8	8	8
1707		8	144*	136	136	1714	8	8 F	128*	136
1710		8	34*	34	95	1716	8	8	8	26
1712		8	8	8	22	1717	8	8	8	136*
1715		8	34*	68	68	1720	8	8	8	232*
1718		8	27	271*	271	1721	8	8	8	8
1719		-	-	-	-	1722 NE	-	-	-	-
1723		8	68*	368*	368	1725	8	8	8	232*
1724		8	8	8	43*	1726	8	8	8	68*
1727		8	8 F	735*	457	1728	8	8	8	41*
1730		8	34*	34	212*	1729	8	8	8	-
1734		8	8	8	271*	1731	34	34	34	34
1735		8	8 F	394*	204	1732	8	8	-	-
1736		8	204*	164	184	1733	8	8	8	8
1737		8	34*	34	58	1739	8	8	204*	204
1738		8	8	8	8	1740	8	8	8	232*
1741	NE	-	-	-	-	1742	8	8	8	8
1743		8	8	8	8	1744 NE	-	-	-	-
1745		8	8	-	-	1746	8	8	394*	407
1747		8	8	8	52*					
GMT		8.4	20.6	45.7	76.6	GMT	9.2	9.3	21	60.2

Table 3 Serum H.I.-antibody titres against B/Hong Kong/8/73

Influvac Spray (n=22)					Placebo (n=21)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		276	306	261	387	1704	6	6	6	6
1702		276	306	191	323	1708	108	230	71	138
1703		255	306	204	187	1709	121	6	16	33
1705		189	108	108	123	1711	6	6	18	19
1706		44	54	54	51	1713	79	6	23	6
1707		127	284	217	284	1714	38	31	31	19
1710		19	19	16	24	1716	18	16	6	18
1712		6	6	18	18	1717	6	25	19	16
1715		48	42	36	74	1720	138	108	108	108
1718		19	32	25	25	1721	6	16	16	6
1719		-	-	-	-	1722 NE	-	-	-	-
1723		116	153	148	204	1725	306	306	306	306
1724		159	159	306	306	1726	16	16	16	21
1727		52	28	59	59	1728	6	6	6	6
1730		306	306	319	612	1729	6	6	18	-
1734		35	16	16	16	1731	58	32	32	32
1735		35	35	49	49	1732	29	20	-	-
1736		16	24	22	22	1733	16	16	6	30
1737		16	284*	284	284	1739	6	6	6	6
1738		22	16	22	16	1740	174	174	174	174
1741	NE	-	-	-	-	1742	6	6	6	6
1743		6	6	6	6	1744 NE	-	-	-	-
1745		6	25	-	-	1746	6	6	6	6
1747		166	166	166	233					

Table 4 Serum H.I. antibody titres against A/Victoria/3/75 of subjects with low initial levels of secretory antibodies (H.I. antibody titre ≤ 16)

Influvac Spray (n-15)						Placebo (n=17)					
Code number	sIgA	I	II	III	IV	Code number	sIgA	I	II	III	IV
1702	12	8	8	8	8	1704	8	34	47	735*	464
1703	12	8	8 F	136*	164	1708	4	8	8 F	232*	68
1705	8	8	68*	68	82	1709	12	8	8	8	41*
1706	8	8	58*	64	64	1713	8	8	8	8	8
1707	1	8	144*	136	136	1714	8	8	8 F	128*	136
1710	8	8	34*	34	95	1716	8	8	8	8	26
1715	8	8	34*	68	68	1717	1	8	8	8	136*
1718	6	8	27	271*	271	1720	2	8	8	8	232*
1727	8	8	8 F	735*	437	1721	8	8	8	8	8
1730	2	8	34	34	212*	1726	10	8	8	8	68*
1735	4	8	8 F	394*	204	1728	2	8	8	8	43*
1737	12	8	34*	34	58	1731	7	34	34	34	34
1738	12	8	8	8	8	1732	5	8	8	-	-
1745	6	8	8	-	-	1733	1	8	8	8	8
1747	4	8	8	8	52*	1740	3	8	8	8	232*
						1742	4	8	8	8	8
						1746	4	8	8	394*	407
GMT		8	20.4	60.4	83.1	GMT		9.5	9.7	21.7	54.2

Table 5 Serum H.I. antibody titres against A/Victoria/3/75 of subjects with low initial levels of secretory antibodies (H.I. antibody titre < 16) and with the exclusion of subjects who were serologically demonstrated to have contracted wild type influenza during the course of the study.

Influvac Spray (n=11)					Placebo (n=13)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	8	8	8	8	1709	8	8	8	41*
1705	8	68*	68	82	1713	8	8	8	8
1706	8	58*	64	64	1716	8	8	8	26
1707	8	144*	136	136	1717	8	8	8	136*
1710	8	34*	34	95	1720	8	8	8	232*
1715	8	34*	68	68	1721	8	8	8	8
1730	8	34*	34	212*	1726	8	8	8	68*
1737	8	34*	34	58	1728	8	8	8	43*
1738	8	8	8	8	1731	34	34	34	34
1745	8	8	-	-	1732	8	8	-	-
1747	8	8	8	52*	1733	8	8	8	8
					1740	8	8	8	232*
					1742	8	8	8	8
GMT	8	25.6	31	53.4	GMT	8.9	8.9	8.9	34.8

Table 6 Serum H.I. antibody titres against A/Victoria of subjects with moderate to high initial levels of secretory antibodies (H.I. antibody titre ≥ 16)

Influvac Spray (n=7)					Placebo (n=4)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	26	34 F	34	34	1711	8	8	8	26
1712	8	8	8	22	1725	8	8	8	232*
1723	8	68*	368*	368	1729	8	8	8	-
1724	8	8	8	43*	1739	8	8	204*	204
1734	8	8	8	271*					
1736	8	204*	164	184					
1743	8	8	8	8					
GMT	9.5	21.2	27	64.7	GMT	8	8	18	107.2

Table 7 Serum H.I. antibody titres against A/Victoria of subjects with moderate to high initial levels of secretory antibodies (H.I.antibody titre $\geq$ 16) with the exclusion of subjects who contracted influenza during the study.

Influvac Spray (n=6)					Placebo (n=3)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	26	34	34	34	1711	8	8	8	26
1712	8	8	8	22	1725	8	8	8	232*
1724	8	8	8	43*	1729	8	8	8	-
1734	8	8	8	271*					
1736	8	204*	164	184					
1743	8	8	8	8					
GMT	9.8	17.5	16.8	48.4	GMT	8	8	8	77.7

If subjects are selected with low levels of secretory antibodies as measured by the Quantitative Haemadsorption test (table 8) and with the exclusion of those subjects who were demonstrated serologically to have contracted wild type influenza virus during the course of the study, the results are as follows:

No subject seroconverted in the placebo group whereas 5/11 subjects seroconverted in the Influvac Spray group i.e. vaccine is more immunogenic in this group (see table 9). The geometric mean titre rose from 8 to 20.1. This is statistically significant (P = 0.05/0.025) After challenge a further 11 subjects seroconverted (7 in the placebo group and 4 in the Spray group).

$$\text{Protection rate} = \left(1 - \frac{4/11}{7/12}\right) \times 100\% = 37.7\%$$

The response to the attenuated challenge vaccine is also significant (P = 0.05) One patient (1730) shows a significant rise twice. Table 10 shows the subjects who have moderate to high levels of secretory IgA (titre $\geq$ 1 QH log 50/ml) and with the influenza sufferers excluded. This time the influvac spray does cause a significant increase in antibody levels (P = 0.1/0.005) The geometric mean titre rises from 10 to 42.

When subjects are chosen with low initial secretory antibody levels as measured by both methods (Table 11) no subject seroconverted in the placebo group whereas 5/8 seroconverted in the influvac spray group. This is statistically significant (P = 0.1/0.05) After challenge a further 8 subjects seroconverted (6 in the placebo group and 2 in the spray group)

$$\text{Protection rate} = \left(1 - \frac{2/8}{6/11}\right) \times 100\% = 54\%$$

When subjects are examined with high initial secretory antibody levels as measured by both methods (table 12) there were too few samples to analyse statistically.

When the subjects, with differing values for initial secretory antibody, are examined the pattern is that if the Quantitative haemadsorption method gives a high value the subjects respond to the influvac spray and are immune to challenge whereas if the Haemagglutination inhibition method gives a high value the subjects show no response to the inactivated influvac spray vaccine but show a good response to the attenuated challenge vaccine.

Table 8 Serum H.I. antibody titres against A/Victoria of subjects with low initial levels of secretory antibodies (Q.H. titre < 1QH log 50/ml)

Influvac Spray (n=15)					Placebo (n=16)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	8	8	8	8	1704	34	47	735*	464
1703	8	8	136*	164	1709	8	8	8	41*
1706	8	58*	64	64	1713	8	8	8	8
1707	8	144*	136	136	1714	8	8	128*	136
1712	8	8	8	22	1717	8	8	8	136*
1715	8	34*	68	68	1720	8	8	8	232*
1718	8	27	271*	271	1721	8	8	8	8
1723	8	68*	368*	368	1725	8	8	8	232*
1724	8	8	8	43*	1726	8	8	8	68*
1730	8	34*	34	212*	1728	8	8	8	41*
1734	8	8	8	271*	1729	8	8	8	-
1735	8	8	394*	204	1731	34	34	34	34
1737	8	34*	34	58	1733	8	8	8	8
1738	8	8	8	8	1740	8	8	8	232*
1747	8	8	8	52*	1742	8	8	8	8
					1746	8	8	394*	407
GMT	8	18.5	39.7	77.2	GMT	9.6	9.8	17.6	61.6

Table 9 Serum H.I. antibody titres against A/Victoria of subjects with low initial levels of secretory antibodies (QH titre < 1 QH log 50/ml) and with the exclusion of subjects who were serologically demonstrated to have contacted wild type influenza during the course of the study.

Influvac Spray (n=11)					Placebo (n=13)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	8	8	8	8	1709	8	8	8	41*
1706	8	58*	64	64	1713	8	8	8	8
1707	8	144*	136	136	1717	8	8	8	136*
1712	8	8	8	22	1720	8	8	8	232*
1715	8	34*	68	68	1721	8	8	8	8
1724	8	8	8	43*	1725	8	8	8	232*
1730	8	34*	34	212*	1726	8	8	8	68*
1734	8	8	8	271*	1728	8	8	8	41*
1737	8	34*	34	58	1729	8	8	8	-
1738	8	8	8	8	1731	34	34	34	34
1747	8	8	8	52*	1733	8	8	8	8
					1740	8	8	8	232*
					1742	8	8	8	8
GMT	8	20.1	21.6	61.5	GMT	8.9	8.9	8.9	41.8

Table 10 Serum H.I. antibody titre against A/Victoria of subjects with moderate to high initial levels of secretory antibodies (QH titre ≥ 1 QH log 50/ml) with the exclusion of subjects serologically demonstrated to have contacted influenza during the study.

Influvac Spray (n=5)					Placebo (n=3)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	26	34	34	34	1711	8	8	8	26
1705	8	68*	68	82	1716	8	8	8	26
1710	8	34*	34	95	1739	8	8	204	204
1736	8	204*	164	184					
1743	8	8	8	8					
GMT	10.1	41.9	40.1	52.3	GMT	8	8	8	26

Table 11 Serum H.I. antibody titres against A/Victoria of subjects with initial low levels of secretory antibodies by both methods of measurement

Influvac Spray (n=8)					Placebo (n=11)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	8	8	8	8	1709	8	8	8	41*
1706	8	58*	64	64	1713	8	8	8	8
1707	8	144*	126	136	1717	8	8	8	136*
1715	8	34*	68	68	1720	8	8	8	232*
1730	8	34*	34	212*	1721	8	8	8	8
1737	8	34*	34	58	1726	8	8	8	68*
1738	8	8	8	8	1728	8	8	8	41*
1747	8	8	8	52*	1731	34	34	34	34
					1733	8	8	8	8
					1740	8	8	8	232*
					1742	8	8	8	8
GMT	8	25.3	27.7	47	GMT	9.1	9.1	9.1	35.6

Table 12 Serum H.I. antibody titres against A/Victoria of subjects with initial high levels of secretory antibodies by both methods of measurement

Influvac Spray (n=3)					Placebo (n=1)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	24	36	34	34	1711	8	8	8	26
1736	8	204*	164	184					
1743	8	8	8	8					

Table 13 Serum H.I. antibody titres of subjects with different initial levels of secretory antibodies by the two methods of measurement

Influvac Spray					Placebo				
Code number	I	II	III	IV	Code number	I	II	III	IV
1. 1705	8	68*	68	82	1716	8	8	8	26
1710	8	34*	34	95					
2. 1712	8	8	8	22	1725	8	8	8	232*
1724	8	8	8	43*					
1734	8	8	8	271*					

1. QH titre: high and HI titre: low

2. HI titre: high and QH titre: low

3.1.2 ANALYSIS OF SPECIFIC SECRETORY ANTIBODY LEVELS

The specific secretory antibody levels by the Haemagglutination-Inhibition test are shown in table 14 and the same group, with the exclusion of those subjects who were serologically demonstrated to have contracted influenza during the course of the study, are shown in table 15.

In the placebo group 4/13 subjects showed a significant rise in titre 6 weeks post vaccination as did 5/17 subjects in the Influvac spray group.

Table 16 shows subjects with low initial levels of secretory H.I. antibodies. Following vaccination with Influvac spray the geometric mean antibody titre rose from 6 to 21.6 ($P = 0.05/0.02$) and in the placebo group the geometric mean titre rose from 4 to 11.2 ($P = 0.02$).

Table 17 shows subjects with moderate to high initial levels of secretory H.I. antibodies. There is little or no response in either group to the initial vaccination however both groups show a slight response to the challenge vaccine. In neither groun is this response significant ($P > 0.5$).

The secretory antibody response corresponds to the serum antibody response when moderate to high initial levels of secretory H.I. antibodies are examined. (cf. Table 7).

Tables 18 through 21 show the secretory antibody titres measured by the Quantitative Haemadsorption test. There is little response to either vaccine or placebo.

Table 14 Secretory H.I. antibody titres against A/Victoria/3/75

Influvac Spray (n=22)						Placebo (n=21)					
Code number		I	II	III	IV	Code number		I	II	III	IV
1701		128	128 F	128	128	1704		8	128* F	128	20
1702		12	12	16	3	1708		4	6 F	32*	128*
1703		12	8 F	48*	24	1709		12	48*	32	24
1705		8	48*	48	48	1711		24	96*	28	128*
1706		8	128*	64	64	1713		8	8	8	4
1707		1	8*	8	4	1714		8	64* F	48	24
1710		8	24	24	80	1716		8	7	8	12
1712		128	56	64	16	1717		1	20	24	128*
1715		8	32*	16	24	1720		2	8	24	48
1718		6	8 F	20	16	1721		8	16	8	8
1719	NE	-	-	-	-	1722	NE	-	-	-	-
1723		32	32	128*	128	1725		48	40	64	22
1724		32	12	12	40	1726		10	16	12	12
1727		8	8 F	8	8	1728		2	48*	12	12
1730		2	32*	32	2	1729		16	16	20	-
1734		24	12	12	40	1731		7	8	12	12
1735		4	16 F	8	96*	1732		5	128*	-	-
1736		96	96	32	96	1733		1	1	1	1
1737		12	3	32	32	1739		16	8 F	2	4
1738		12	128*	16	32	1740		3	12	2	24*
1741	NE	-	-	-	-	1742		4	8	8	3
1743		24	24	16	24	1744	NE	-	-	-	-
1745		6	4	-	-	1746		4	6 F	8	5
1747		4	4	4	8						
GMT		12.2	20.3	23.2	25.4	GMT		6.1	18	13.3	15.1

Table 15 Secretory H.I. antibody titres against A/Victoria with the exclusion of subjects who were serologically demonstrated to have contracted influenza during the study

Influvac Spray (n=17)					Placebo (n=16)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	128	128	128	128	1709	12	48*	32	24
1702	12	12	16	3	1711	24	96*	28	128*
1705	8	48*	48	48	1713	8	8	8	4
1706	8	128*	64	64	1716	8	8	8	12
1707	1	8	8	4	1717	1	20	24	128*
1710	8	24	24	80	1720	2	8	24	48
1712	128	56	64	16	1721	8	16	8	8
1715	8	32*	16	24	1725	48	40	64	22
1724	32	12	12	40	1726	10	16	12	12
1730	2	32*	32	2	1728	2	48*	12	12
1734	24	12	12	40	1729	16	16	20	-
1736	96	96	32	96	1731	7	8	12	12
1737	12	3	32*	32	1732	5	128*	-	-
1738	12	128*	16	32	1733	1	1	1	1
1743	24	24	16	24	1740	3	12	2	24
1745	6	4	-	-	1742	4	8	8	3
1747	4	4	4	8					
GMT	13	23.6	23	23.4	GMT	5.9	16.6	11.6	14.6

Table 16 Secretory H.I. antibody titres against A/Victoria of subjects with low initial levels (H.I. antibody titre ≤ 16) and influenza sufferers excluded

Influvac Spray (n=11)					Placebo (n=12)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	12	12	16	3	1709	12	48*	32	24
1705	8	48*	48	48	1713	8	8	8	4
1706	8	128*	64	64	1716	8	8	8	12
1707	1	8	8	4	1717	1	20*	24	128*
1710	8	24	24	80	1720	2	8	24	18
1715	8	32*	16	24	1721	8	16	8	8
1730	2	32*	32	2	1726	10	16	12	12
1737	12	3	32	32	1728	2	48*	12	12
1738	12	128*	16	32	1731	8	8	12	12
1745	6	4	-	-	1733	1	1	1	1
1747	4	4	4	8	1740	3	12	2	24
					1742	4	8	8	3
GMT	6	21.6	19.9	16	GMT	4.1	11.2	8.9	11.8

Table 17 Secretory H.I. antibody titres against A/Victoria of subjects with moderate initial levels (H.I. antibody titre $\geq$ 16) and influenza sufferers excluded

Influvac Spray (n=6)					Placebo (n=3)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	128	128	128	128	1711	24	96*	28	128*
1712	128	56	64	16	1725	48	40	64	22
1724	32	12	12	40	1729	16	16	20	-
1734	24	12	12	40					
1736	96	96	32	96					
1743	24	24	16	24					
GMT	36.6	36.5	29.1	44.3	GMT	26.4	39.5	33	53

Table 18 Secretory antibody titres against A/Victoria/3/75 by the Quantitative Haemadsorption Test (QH log 50/ml)

Influvac Spray (n=20)					Placebo (n=21)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		2.52	2.62 F	2.52	2.82	1704	0.50	1.60*	-	-
1702		0.50	0.50	0.69	0.50	1708	0.50	0.50 F	0.99	1.95*
1703		0.50	0.50 F	1.11*	0.91	1709	0.50	0.50	0.90	0.50
1705		1.60	1.80	1.80	1.80	1711	1.44	1.40	1.49	1.57
1706		0.89	1.86*	1.40	1.72	1713	0.50	0.78	0.50	0.50
1707		0.50	1.79*	1.48	1.48	1714	0.50	1.16*F	1.38	0.50
1710		1.72	1.42	1.72	1.23	1716	1.20	1.28	1.35	1.13
1712		0.50	0.50	0.50	0.50	1717	0.50	0.50	1.23	1.28
1715		0.85	0.78	1.20	1.73	1720	0.50	0.50	0.50	0.50
1718		0.50	0.50	0.85	1.08	1721	0.50	0.50	0.50	0.50
1719	NE	-	-	-	-	1722 NE	-	-	-	-
1723		1.65	2.45*	1.95	2.20	1725	0.50	0.50	0.50	0.50
1724		0.50	0.50	0.50	0.50	1726	0.50	0.50	0.50	0.50
1727		-	- F	-	-	1728	0.50	1.34*	0.50	0.50
1730		0.50	0.50	0.50	0.50	1729	0.50	0.78	0.50	-
1734		0.50	0.63	0.74	0.74	1731	0.50	1.12*	1.16	0.50
1735		0.50	0.75 F	0.50	1.70	1732	0.50	1.41*	-	-
1736		1.17	0.50	1.12	1.48	1733	0.50	0.50	0.50	1.20*
1737		0.93	0.93	1.03	1.20	1739	1.00	1.05	0.50	0.50
1738		0.50	2.56*	1.21	1.56	1740	0.85	0.89	0.50	0.50
1741	NE	-	-	-	-	1742	0.50	0.50	0.50	0.50
1743		1.15	1.28	1.27	0.50	1744 NE	-	-	-	-
1745		-	-	-	-	1746	0.50	0.85	0.50	0.50
1747		0.50	0.50	0.50	0.50					

Note: 4 fold rise in titre ≡ a difference of 0.6 QH log 50/ml.

Table 19 Secretory antibody titres against A/Victoria by the Quantitative Haemadsorption Test (QH log 50/ml) with the exclusion of influenza sufferers

Influvac Spray (n=17)					Placebo (n=16)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	2.52	2.62	2.52	2.82	1709	0.50	0.50	0.90	0.50
1702	0.50	0.50	0.69	0.50	1711	1.44	1.40	1.49	1.57
1705	1.60	1.80	1.80	1.80	1713	0.50	0.78	0.50	0.50
1706	0.89	1.86*	1.40	1.72	1716	1.20	1.28	1.35	1.13
1707	0.50	1.79*	1.48	1.48	1717	0.5	0.5	1.23	1.28
1710	1.72	1.42	1.72	1.23	1720	0.5	0.5	0.5	0.5
1712	0.50	0.50	0.50	0.50	1721	0.5	0.5	0.5	0.5
1715	0.85	0.78	1.20	1.73	1725	0.5	0.5	0.5	0.5
1724	0.50	0.50	0.50	0.50	1726	0.5	0.5	0.5	0.5
1730	0.50	0.50	0.50	0.50	1728	0.5	1.34*	0.5	0.5
1734	0.50	0.63	0.74	0.74	1729	0.5	0.78	0.5	-
1735	0.50	0.75	0.50	1.7	1731	0.5	1.12*	1.16	0.5
1736	1.17	1.50	1.12	1.48	1732	0.5	1.41*	-	-
1737	0.93	0.93	1.03	1.20	1733	0.5	0.5	0.5	1.2*
1738	0.50	2.56*	1.21	1.56	1740	0.85	0.89	0.5	0.5
1743	1.15	1.28	1.27	0.5	1742	0.5	0.5	0.5	0.5
1747	0.50	0.50	0.50	0.5					
GMT	0.9	1.2	1.1	1.2	GMT	0.6	0.9	0.8	0.8

Table 20 Secretory antibody titres against A/Victoria by the Quantitative Haemadsorption Test of subjects with low initial levels (≤ 1 QH log 50/ml) and with the exclusion of influenza sufferers

Influvac Spray (n=9)					Placebo (n=13)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1702	0.5	0.5	0.69	0.5	1709	0.5	0.5	0.9	0.5
1707	0.5	1.79*	1.48	1.48	1713	0.5	0.78	0.5	0.5
1712	0.5	0.5	0.5	0.5	1717	0.5	0.5	1.23	1.28
1724	0.5	0.5	0.5	0.5	1720	0.5	0.5	0.5	0.5
1730	0.5	0.5	0.5	0.5	1721	0.5	0.5	0.5	0.5
1734	0.5	0.63	0.74	0.76	1725	0.5	0.5	0.5	0.5
1735	0.5	0.75	0.5	1.7*	1726	0.5	0.5	0.5	0.5
1738	0.5	2.56*	1.21	1.56	1728	0.5	1.34*	0.5	0.5
1747	0.5	0.5	0.5	0.5	1729	0.5	0.78	0.5	-
					1731	0.5	1.12*	1.16	0.5
					1732	0.5	1.41*	-	-
					1733	0.5	0.5	0.5	1.2
					1742	0.5	0.5	0.5	0.5
GMT	0.5	0.9	0.7	0.9	GMT	0.5	0.7	0.65	0.63

Table 21 Secretory antibody titres against A/Victoria by the Quantitative Haemadsorption Test of subjects with moderate to high initial levels (≥ 1 QH log 50/ml) and with the exclusion of influenza sufferers

Influvac Spray (n=8)					Placebo (n=3)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	2.52	2.62	2.52	2.82	1711	1.44	1.40	1.49	1.57
1705	1.60	1.8	1.8	1.8	1716	1.20	1.28	1.35	1.13
1706	0.89	1.86*	1.4	1.72	1740	0.85	0.89	0.5	0.5
1710	1.72	1.42	1.72	1.23					
1715	0.85	0.78	1.2	1.73					
1736	1.17	1.5	1.12	1.48					
1737	0.93	0.93	1.03	1.20					
1743	1.15	1.28	1.27	0.5					
GMT	1.35	1.52	1.51	1.56	GMT	1.16	1.19	1.11	1.07

3.1.3 ANALYSIS OF TOTAL LOCAL ANTIBODY LEVELS

Total local IgG and IgA antibody levels were measured by Radioimmunoassay and the results shown in tables 22 and 23 . The results confirm the presence of local IgA and IgG. The base-line antibody levels exhibit the expected ratio of IgA:IgG (ie 5:1).

Figure 5 shows the secretory antibody levels measured both by the Quantitative Haemadsorption test and by the Haem-agglutination Inhibition test compared with levels of total local IgA measured by Radioimmunoassay. To facilitate analysis the results have been divided into three categories: Influvac spray group, Placebo group and subjects naturally infected by influenza virus.

All three groups show a response to the initial stimulus. The two trial groups show a fall in the four month period between specimens II and III, the Influvac spray group showing a less pronounced fall than the placebo group, whereas the group which were naturally infected shows a "holding of level". When measured by the Haemagglutination Inhibition Test this group shows a rise. Following challenge all three groups show a small rise.

3.1.4 ANALYSIS OF TOTAL SERUM IgA and IgM ANTIBODY LEVELS

Total serum IgA and IgM antibody levels were measured by single radial immunodiffusion and the results are shown in tables 24 and 25 .

The range of base-line serum IgA was 90 - 500 mg/dl with a mean of 254 mg/dl and a standard deviation of 93 mg/dl. The coefficient of variation was 4.3%.

The range of base-line IgM concentrations was found to be 36 - 210 mg/dl with a mean of 108 mg/dl and a standard deviation of 43 mg/dl. The coefficient of variation was 6.5%.

3.1.5 ANALYSIS OF SPECIFIC SERUM IgG ANTIBODY LEVELS

Specific serum IgG against Influenza A/Victoria/3/75 was measured by the Fluorescent Antibody Technique and the results are shown in table 26.

These results indicate that this technique is useful for detecting infection. It does not, however, appear to detect antibody changes produced by the vaccine. (cf table 2).

Table 22 Total local IgG (mg/dl) by Radioimmunoassay

Influvac Spray (n=22)					Placebo (n=21)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		5.00	3.79	2.78	4.13	1704	0.40	0.71	5.00	2.19
1702		0.04	0.03	0.03	0.06	1708	0.14	0.59	0.26	0.73
1703		1.29	0.42	2.94	1.67	1709	0.43	1.15	1.00	0.89
1705		0.04	0.12	0.40	0.43	1711	0.56	0.41	0.77	0.83
1706		1.31	5.00	3.86	2.37	1713	0.81	1.51	0.82	0.85
1707		1.59	0.97	1.33	1.06	1714	2.14	1.19	0.56	0.61
1710		0.23	0.46	2.88	4.00	1716	0.09	0.16	0.22	0.49
1712		5.00	5.00	5.00	1.25	1717	0.06	0.49	1.74	0.59
1715		0.59	0.12	0.48	1.51	1720	0.17	0.12	0.75	1.00
1718		0.22	0.16	0.07	0.29	1721	0.10	0.23	0.21	0.28
1719		-	-	-	-	1722 NE	-	-	-	-
1723		0.30	2.51	1.37	0.34	1725	0.20	0.36	0.38	0.24
1724		0.11	0.04	0.05	0.07	1726	0.17	2.09	0.25	1.35
1727		0.74	0.34	0.40	0.34	1728	0.50	1.00	0.92	1.26
1730		0.08	0.19	0.17	0.03	1729	1.32	2.75	3.34	-
1734		1.09	0.41	0.35	1.84	1731	0.15	0.23	0.35	0.43
1735		0.11	0.10	0.22	1.38	1732	0.71	5.00	-	-
1736		0.62	1.54	0.33	0.50	1733	5.00	1.52	3.16	0.84
1737		1.68	0.54	0.76	0.67	1739	0.09	0.16	0.11	0.34
1738		0.80	1.63	0.48	1.84	1740	0.09	0.32	0.07	0.59
1741	NE	-	-	-	-	1742	0.17	0.83	0.98	1.24
1743		0.38	0.52	0.25	0.44	1744 NE	-	-	-	-
1745		0.40	0.15	-	-	1746	0.20	0.62	0.49	0.28
1747		0.06	0.36	0.04	0.61					

Table 23 Total local IgA (mg/dl) by Radioimmunoassay

Influvac Spray (n=22)					Placebo (n=21)				
Code number	I	II	III	IV	Code number	I	II	III	IV
1701	28.74	23.00	35.68	75.42	1704	1.15	10.55	15.00	4.45
1702	1.89	1.33	3.46	0.35	1708	1.39	3.39	4.85	6.26
1703	6.48	7.41	15.00	9.55	1709	4.15	8.97	6.31	5.57
1705	0.86	1.65	3.56	1.90	1711	2.99	8.21	4.99	15.00
1706	1.71	13.06	5.30	26.42	1713	3.85	4.20	3.96	2.10
1707	1.58	2.44	1.67	1.88	1714	7.31	9.15	8.08	5.89
1710	2.39	5.66	4.44	8.79	1716	2.18	1.27	1.67	3.21
1712	15.00	7.72	6.29	4.31	1717	0.32	1.10	4.04	4.18
1715	0.74	1.42	1.39	3.05	1720	1.20	1.79	4.30	7.64
1718	1.76	2.64	2.85	5.08	1721	0.66	1.55	0.94	1.29
1719	-	-	-	-	1722 NE	-	-	-	-
1723	3.97	15.00	5.28	3.73	1725	3.04	3.09	2.78	0.89
1724	3.02	0.58	0.62	1.91	1726	1.99	4.12	1.31	2.43
1727	1.56	1.08	0.99	1.12	1728	0.56	7.89	1.52	4.03
1730	1.13	2.09	1.29	0.24	1729	3.10	5.19	4.14	-
1734	4.48	1.62	2.91	6.17	1731	2.38	2.99	2.59	3.03
1735	0.43	0.93	0.84	8.05	1732	1.90	15.00	-	-
1736	8.66	13.14	2.58	8.57	1733	10.68	3.19	6.62	2.54
1737	4.04	0.76	6.12	4.62	1739	6.16	4.85	2.87	3.47
1738	5.89	15.00	8.41	15.00	1740	0.66	3.28	0.78	3.05
1741 NE	-	-	-	-	1742	2.05	3.49	4.20	1.83
1743	10.58	6.76	4.33	8.69	1744 NE	-	-	-	-
1745	2.99	0.97	-	-	1746	1.60	3.15	1.91	1.41
1747	0.65	0.61	0.64	1.19					

Figure 5. Comparison of specific and local antibody levels.

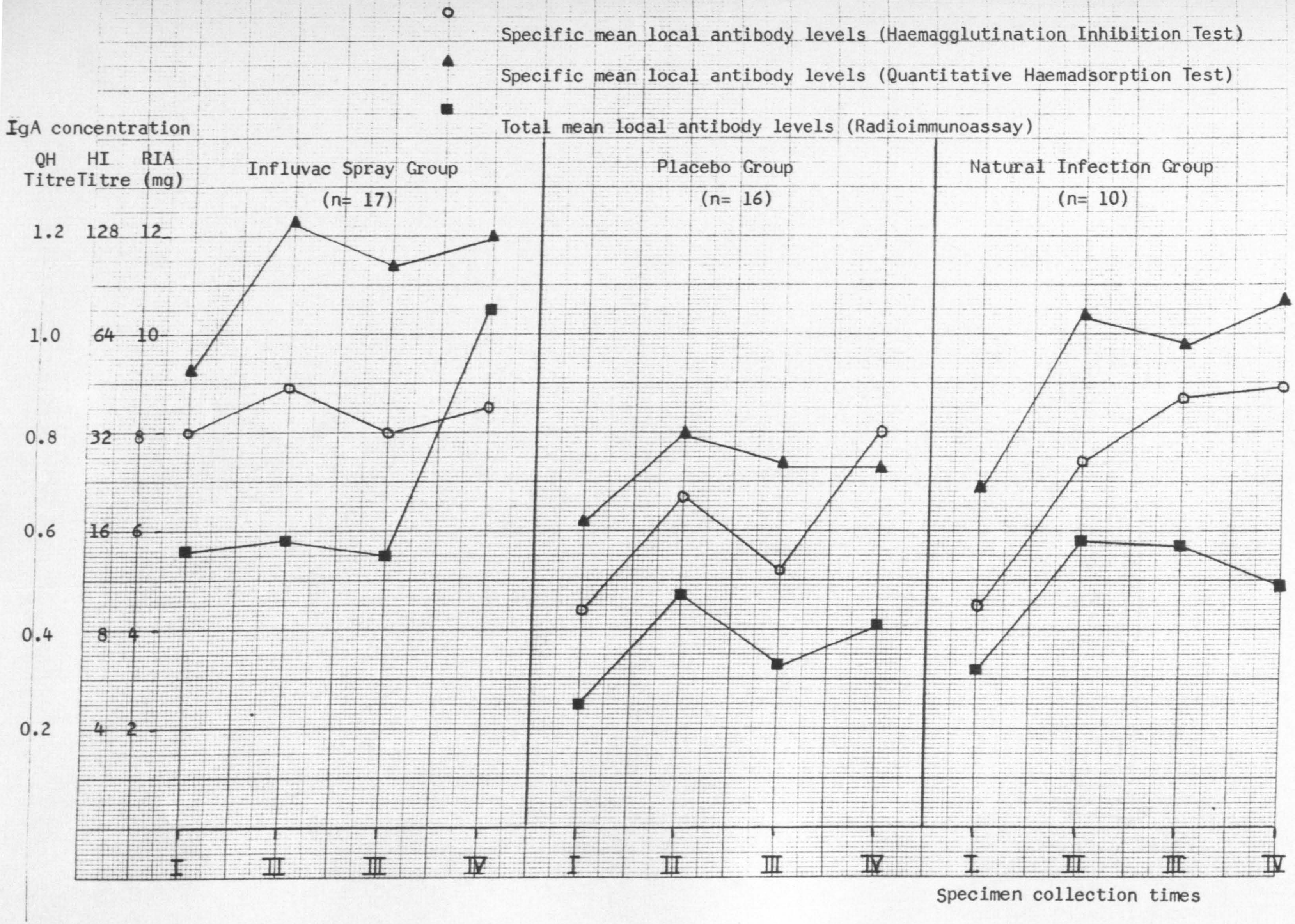


Table 24 Serum IgA levels by Single Radial Immunodiffusion (mg/dl)

Influvac Spray (n=20)					Placebo (n=19)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		190	165	275	195	1704	215	140	205	190
1702		160	150	140	150	1708	270	215	267	267
1703		125	125	125	125	1709	345	251	345	327
1705		108	108	74	117	1711	251	251	167	350
1706		260	251	327	377	1713	274	284	304	304
1707		167	123	177	177	1714	310	310	395	320
1710		270	280	315	345	1716	228	191	238	238
1712		263	263	227	263	1717	365	455	500	470
1715		160	152	229	190	1720	304	274	424	294
1718		211	150	185	235	1721	189	152	132	167
1719		-	-	-	-	1722 NE	-	-	-	-
1723		340	390	380	500	1725	231	189	305	351
1724		131	178	153	131	1726	242	242	285	272
1727		262	262	235	212	1728	390	390	390	390
1730		435	327	340	450	1729	-	-	-	-
1734		500	500	500	500	1731	212	167	142	160
1735		90	80	80	80	1732	-	-	-	-
1736		160	105	150	150	1733	242	235	250	327
1737		225	295	340	340	1739	240	247	255	320
1738		168	152	152	168	1740	385	385	385	385
1741 NE		-	-	-	-	1742	385	227	320	445
1743		225	235	305	242	1744 NE	-	-	-	-
1745		-	-	-	-	1746	360	-	-	-
1747		-	-	-	-					
GMT		202	192	210	218	GMT	279	244	278	297

Coefficient of Variation = 4.3%

Table 25 Serum IgM levels by Single Radial Immunodiffusion (mg/dl)

Influvac Spray (n=21)					Placebo (n=20)				
Code number		I	II	III	IV	Code number	I	II	III	IV
1701		36	30	36	36	1704	93	71	100	103
1702		46	46	40	46	1708	158	132	116	132
1703		84	84	84	84	1709	93	93	116	116
1705		118	103	103	126	1711	78	78	51	100
1706		71	64	93	85	1713	92	100	115	107
1707		64	93	44	85	1714	78	64	78	78
1710		124	124	132	108	1716	210	152	186	210
1712		95	95	61	95	1717	116	116	182	149
1715		100	95	123	124	1720	170	153	170	145
1718		210	158	194	184	1721	43	43	43	57
1719		-	-	-	-	1722	NE -	-	-	-
1723		116	124	124	124	1725	69	43	32	53
1724		119	103	88	88	1726	93	101	93	109
1727		124	124	124	92	1728	111	111	95	87
1730		150	150	150	111	1729	50	50	50	-
1734		68	64	64	64	1731	109	92	103	142
1735		166	124	184	200	1732	-	-	-	-
1736		184	177	159	116	1733	59	59	67	74
1737		98	55	34	34	1739	116	116	116	116
1738		145	134	142	142	1740	80	70	70	70
1741	NE	-	-	-	-	1742	141	109	140	144
1743		132	132	132	132	1744	NE -	-	-	-
1745		-	-	-	-	1746	92	92	92	92
1747		135	127	127	127					
GMT		104	97	94	96	GMT	95	86	91	104

Coefficient of Variation = 6.5%

Table 26 Serum IgG titres against A/Victoria/3/75 by the Fluorescent Antibody Technique

Influvac Spray (n=22)					Placebo (n=21)				
Code number	I	II		III	IV	Code number	I	II	III	IV
1701	<16	<16	F	<16	<16	1704	<16	<16	<16	<16
1702	<16	<16		<16	<16	1708	<16	<16	F 1:32*	<16
1703	<16	<16	F	1:64*	1:64	1709	<16	<16	<16	<16
1705	<16	<16		<16	<16	1711	<16	<16	<16	<16
1706	<16	<16		<16	<16	1713	<16	<16	<16	<16
1707	<16	<16		1:32*	1:32	1714	<16	<16	F 1:64*	1:64
1710	<16	<16		<16	<16	1716	1:16	1:16	1:16	1:16
1712	<16	<16		<16	<16	1717	<16	<16	<16	<16
1715	<16	<16		<16	<16	1720	<16	<16	<16	<16
1718	<16	<16		1:32*	1:32	1721	<16	<16	<16	<16
1719	-	-		-	-	1722 NE	-	-	-	-
1723	<16	<16		1:32*	1:32	1725	<16	<16	<16	<16
1724	<16	<16		1:64*	1:64	1726	<16	<16	<16	<16
1727	<16	<16	F	1:64*	1:64	1728	<16	<16	<16	<16
1730	<16	<16		<16	<16	1729	<16	<16	<16	1:32*
1734	<16	<16		<16	<16	1731	<16	<16	<16	<16
1735	<16	<16	F	1:64*	<16	1732	<16	<16	-	-
1736	<16	<16		<16	<16	1733	<16	<16	<16	<16
1737	<16	<16		<16	<16	1739	<16	<16	<16	<16
1738	<16	<16		<16	<16	1740	1:32	1:32	1:32	1:32
1741 NE	-	-		-	-	1742	<16	<16	<16	<16
1743	<16	<16		<16	<16	1744 NE	-	-	-	-
1745	<16	<16		-	-	1746	<16	<16	<16	<16
1747	<16	<16		<16	<16					

3.2 GENERAL PRACTICE STUDY

3.2.1 IDENTIFICATION OF RESPIRATORY PATHOGENS.

These results are shown in tables 27, 28 and figures 6 to 9.

Winter 1 (November, 1977- April, 1978).

146 patients were included in the survey and 292 swabs were examined. 125 swabs from 96 patients (65%) yielded bacteria and 21 swabs from 149 patients (7%) gave viruses. 11 swabs (6%) gave both bacterial and viral growth.

The majority of viruses (81%) were isolated from the first swab. Bacteria were isolated from both swabs in 21% of patients. Of these, 37% had the same bacteria isolated from each swab and in 63%, the bacteria isolated were different. In 37% of patients neither bacteria nor viruses were grown. This high failure rate might be due to the swab being taken at too late a stage in the infection, the type of infecting agent not being within the range screened for (eg. certain types of Rhinovirus), or an alternative causative agent (eg. respiratory allergy).

Some of the most commonly isolated bacteria have doubtful pathogenicity. A comparison was made between the bacteria isolated from nasal swabs taken from staff at Belvidere Hospital and swabs taken from the General Practice patients. The hospital staff, with an age range of 16 to 60 years,presented at the Hospital " Staff Health Department " with an assortment of ailments, not necessarily respiratory. In this group, Staphylococcus aureus and Staphylococcus albus were most commonly isolated. In the General Practice cases there was, in addition to the bacteria mentioned, an increase in the isolation rate of Diplococcus pneumonia, Haemophilus influenza, Streptococcus pyogenes and Candida species.

Winter 2. (November, 1978- April, 1979)

93 patients took part in the 2nd. year of the survey and 93 swabs were examined. It was decided to concentrate on Viral studies on the 1st. specimen only and no Bacteriology was carried out.

The virus isolation rate was 12%.

The viral studies show that, during both winters, Influenza Type A virus was the most common respiratory pathogen identified during the periods of the study. The low isolation rate for Respiratory Syncytial virus (R.S.V.) probably reflects the relatively small number of infants encountered in the General Practice situation compared to the hospital population. There was difficulty in finding an alternative host cell line to Rhesus Monkey Kidney for the isolation of R.S.V. (Rhesus monkey exports from India have now been banned. As a result kidney cells from these monkeys, which were for many years the main cell line used to show respiratory viruses, are no longer available.)

The viruses isolated are presented in figures 6 to 9. Charts have also been included to show how these results compare with laboratory evidence of viral infections at the Virus Laboratory, Belvidere Hospital, Glasgow and throughout Scotland. It should be noted that the latter two groups include viruses identified by serological techniques.

Table 27 - Laboratory Evidence of Bacterial Infections - Winter 1.

Total number of swabs examined = 292

Total number of subjects = 146

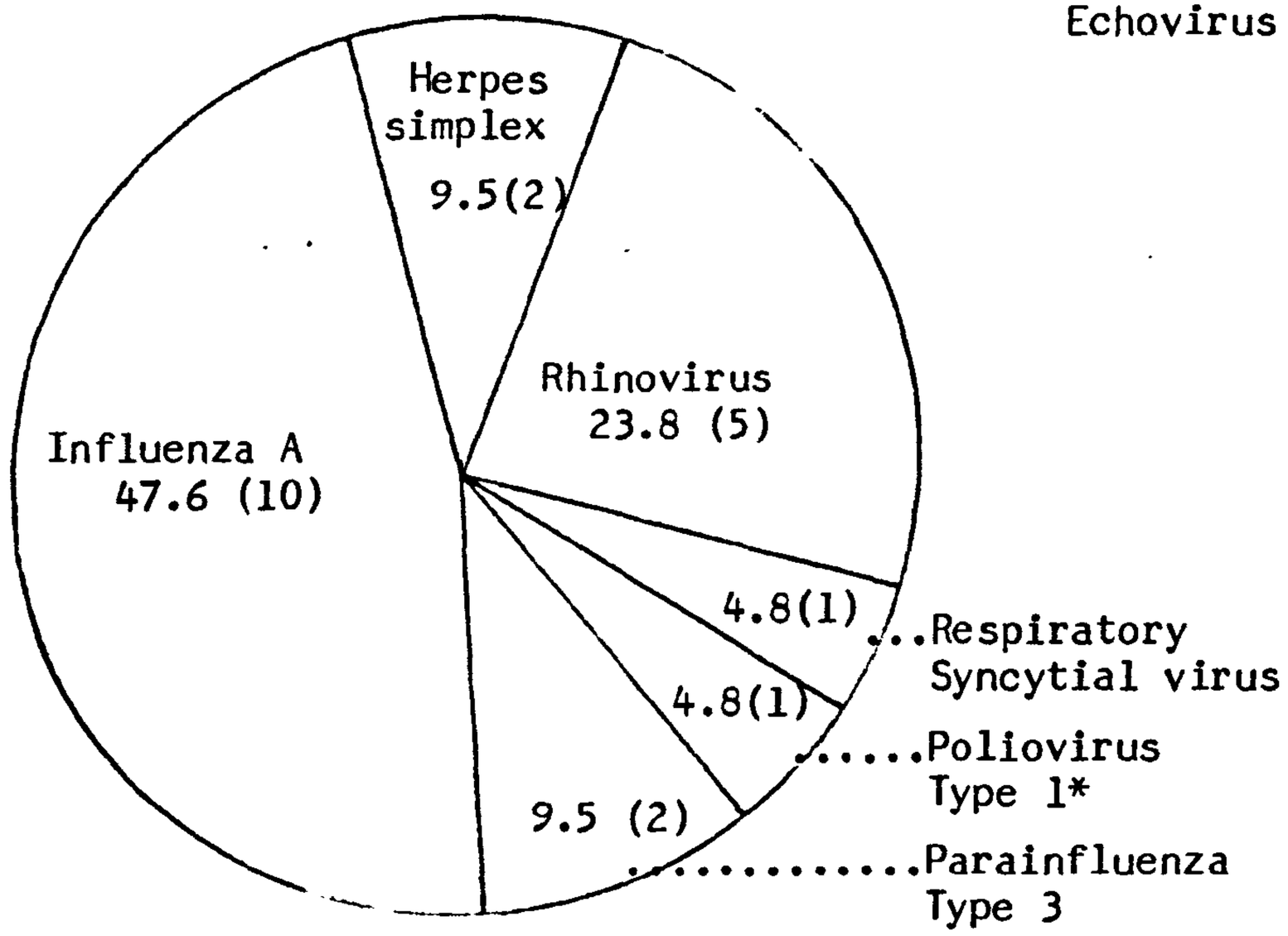
Isolate	No. of Isolates
Staph. aureus	30
Staph. albus	27
Neisseria catarrhalis	2
Dip. pneumoniae	15
Strep. viridans	9
Strep. pyogenes (Lancefield A)	2
Haemophilus influenza	13
Candida sp.	15
Commensal diptheroids	14
No growth obtained	108
Not examined	57
	292

TABLE 28. A comparison of bacterial isolates obtained from nasal swabs from hospital staff, and specimens of nasal swabs from patients in a general practice.

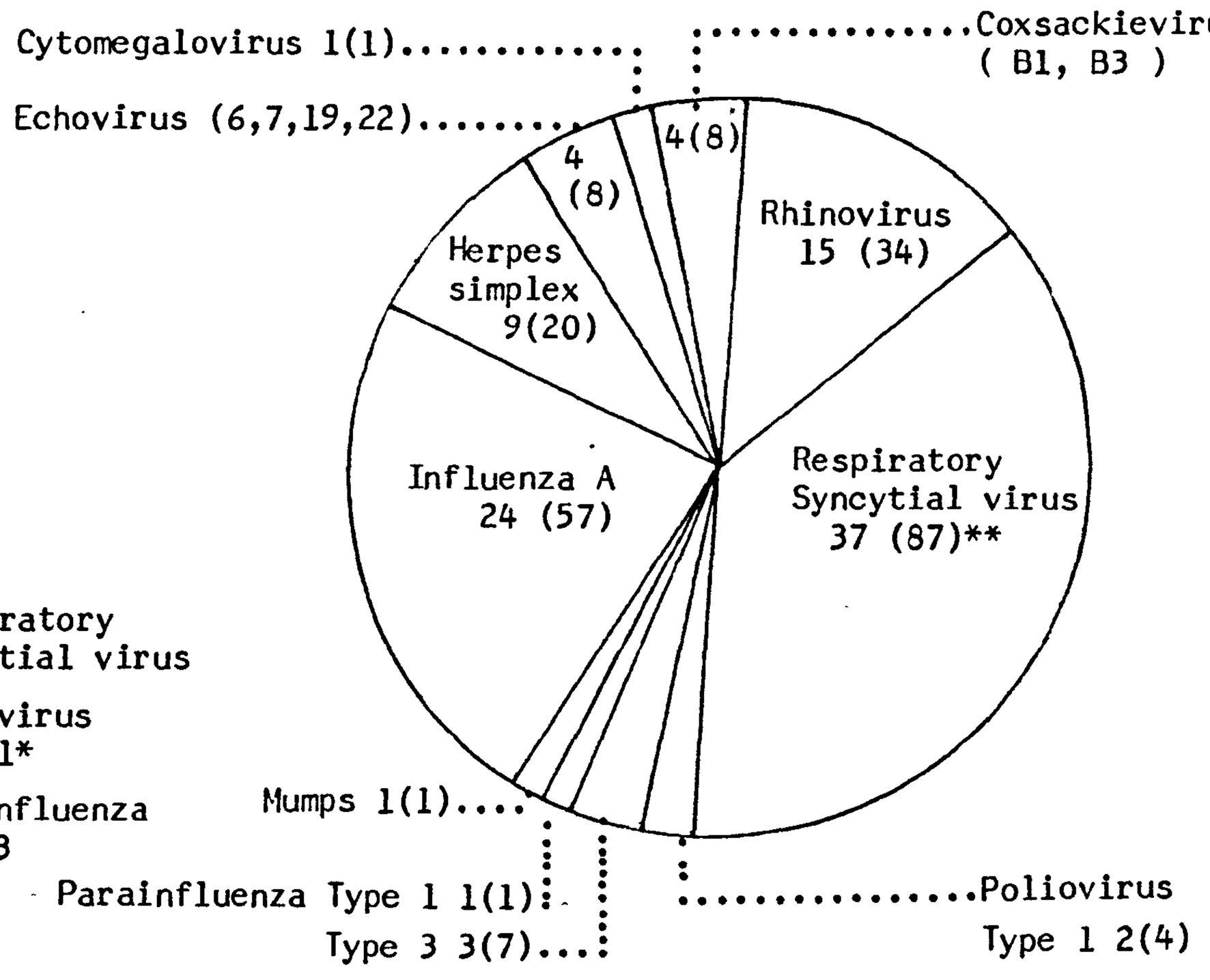
Isolate	Hospital		General Practice	
	No. of isolates	(% total)	No. of isolates	(% total)
Staph. aureus	23	(23)	7	(9.3)
Staph. albus	50	(50)	8	(10.6)
Neisseria catarrhalis	2	(2)	4	(5.3)
Dip. pneumoniae	2	(2)	9	(12.0)
H. influenzae	-	-	5	(6.6)
Diptheroids	5	(5)	1	(1.3)
Strep. viridans	4	(4)	1	(1.3)
Strep. pyogenes	-	-	2	(2.6)
Klebsiella sp.	1	(1)	-	-
Proteus sp.	2	(2)	-	-
Candida sp.	-	-	6	(8.0)
No growth	15	(15)	32	(42.6)
	104		75	

Figure 6. Laboratory Evidence of Viral Infections (Winter 1).

1. General Practice Study.
(Total no. of isolates = 21)



2. Belvidere Hospital Laboratory.
(Total no. of isolates = 237)



* probably a vaccine strain

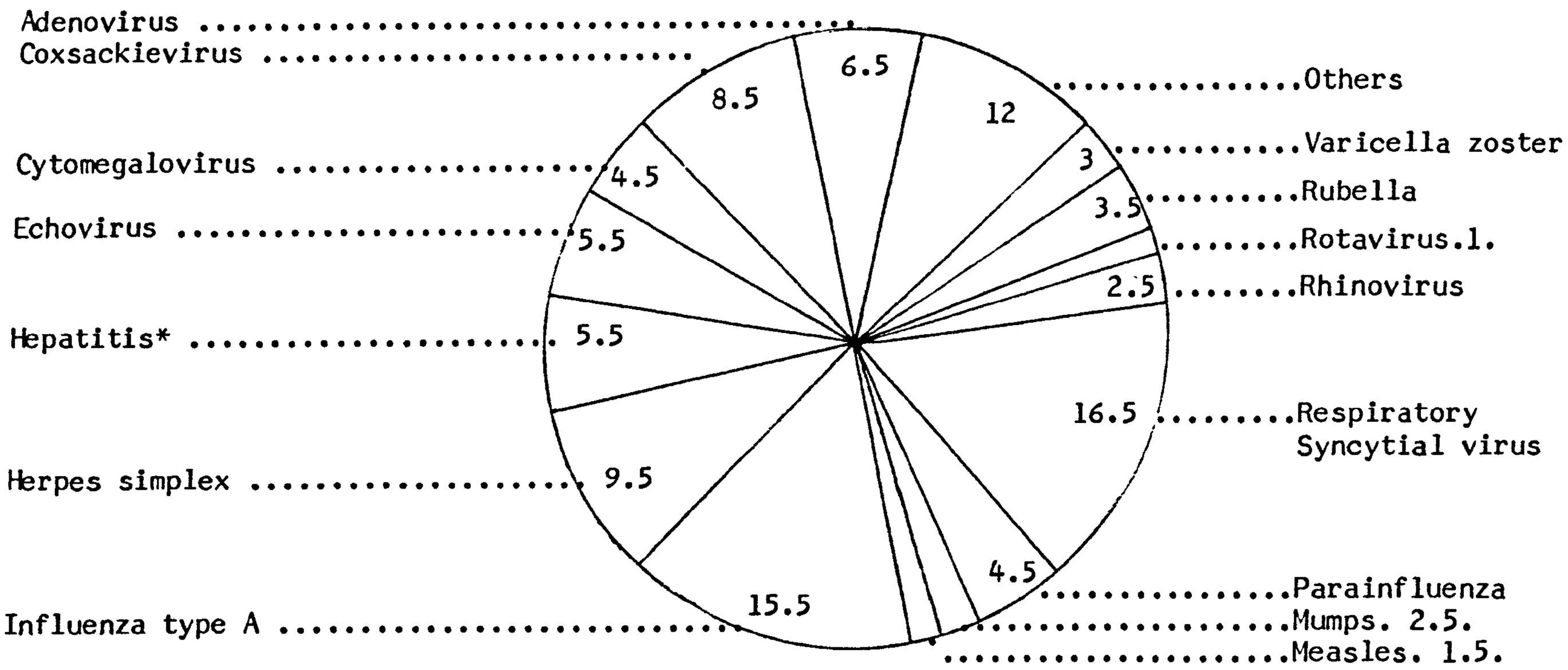
Total no. of patients = 146
Isolation rate = 14.5%

** mostly hospitalised paediatric patients

Total number of patients = 735
Isolation rate = 32%

Figures in these charts represent the percentage of the total number of viruses identified.
The numbers of viruses identified are given in parenthesis.

Figure 7. Laboratory Evidence of Viral Infections in Scotland (Winter 1).



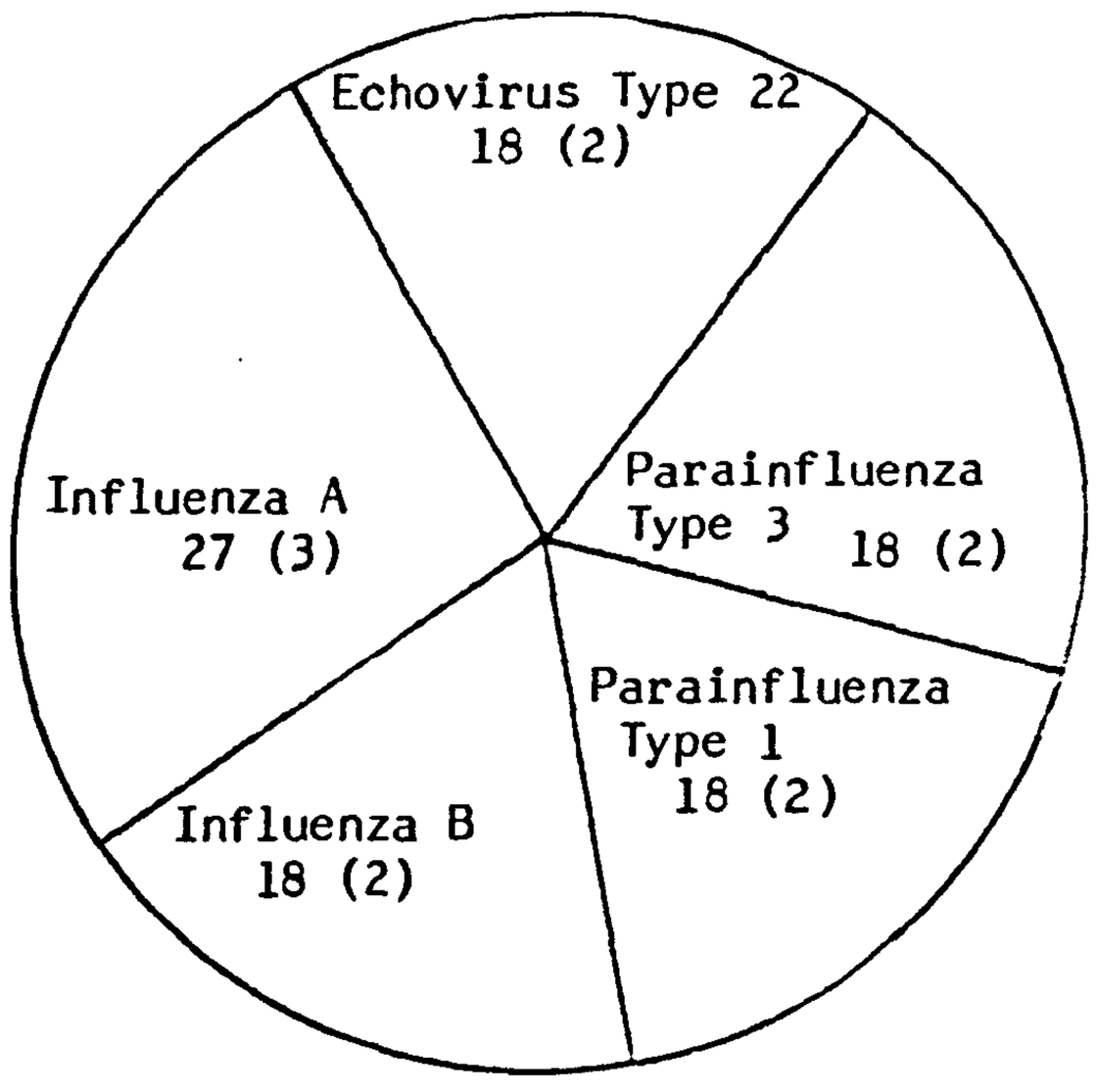
* Not tested for at Belvidere Hospital.

Figures in this chart represent the percentage of the total number of Viruses reported.

(Data from C.D.S. Unit, Ruchill Hospital, Glasgow.)

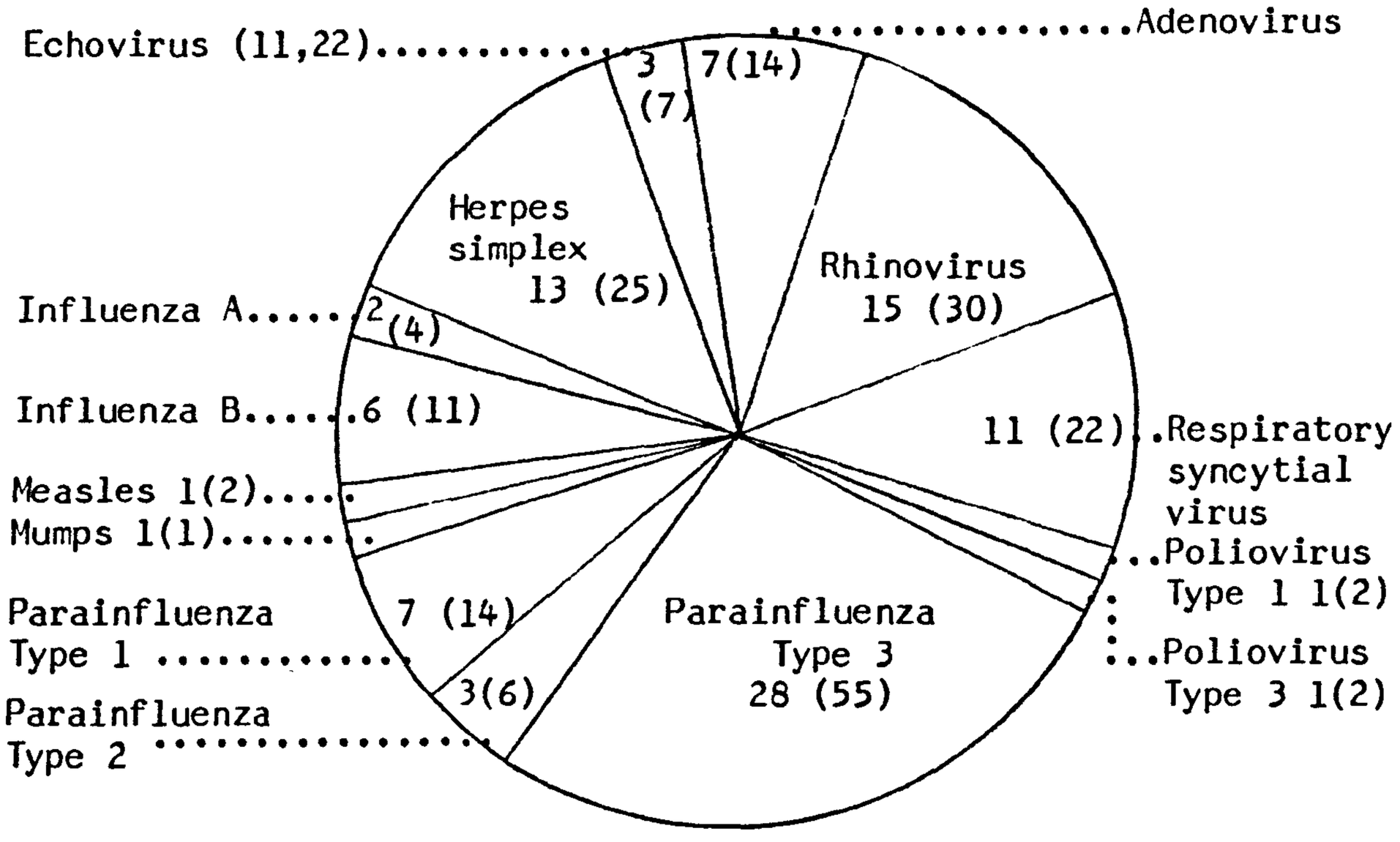
Figure 8. Laboratory Evidence of Viral Infections (Winter 2).

1. General Practice Study.
(Total no. of isolates = 11)



Total no. of patients = 93
Isolation rate = 12%

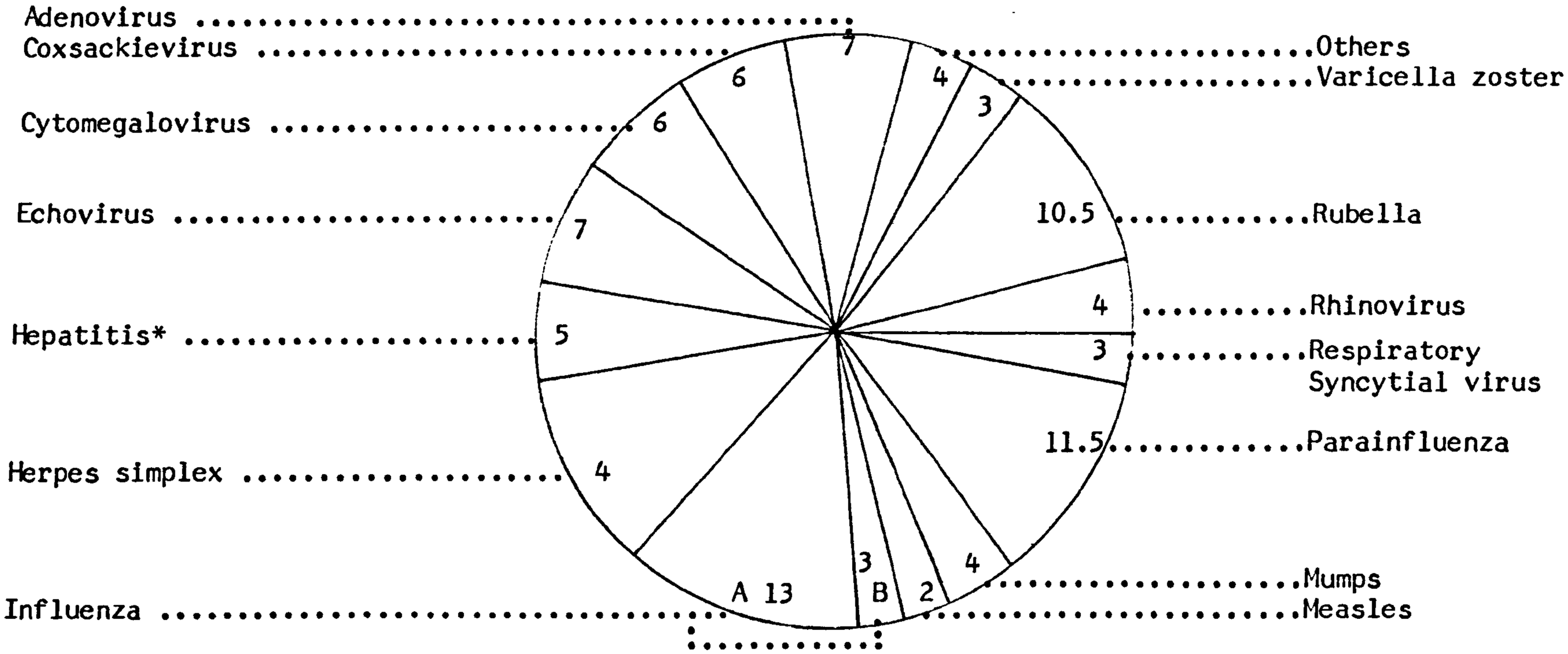
2. Belvidere Hospital Laboratory.
(Total no. of isolates = 195)



Total no. of patients = 798
Isolation rate = 24%

Figures in these charts represent the percentage of the total number of viruses identified.
The numbers of viruses identified are given in parenthesis.

Figure 9. Laboratory Evidence of Viral Infections in Scotland (Winter 2).



* Not tested for in Belvidere Hospital.

Figures in this chart represent the percentage of the total number of viruses reported.

(Data from C.D.S. Unit, Ruchill Hospital, Glasgow.)

3.2.2 ANALYSIS OF SECRETORY IgA PRODUCTION

In Winter 1, 92 patients and in Winter 2, 54 patients were examined by Rocket Electrophoresis (Plate 1). All relevant information about each patient has been presented in table 29.

In both study periods the amount of sIgA found varied significantly between the acute and convalescent phase specimens during respiratory infection (Winter 1, P = 0.05/ 0.025 and Winter 2, P = 0.0005).

The range of sIgA was from 0 - 11mm. (rocket height) which is approximately 0 - 250mg/dl.

In Winter 1, 24 specimens had undetectable sIgA in at least one specimen whereas in Winter 2, sIgA was present in all specimens.

Six patients had no detectable sIgA in either acute or convalescent specimens in the first winter. Serum specimens were taken from 4 of these patients and they were found to have normal levels of serum IgG, IgA and IgM. Unfortunately it was not possible to obtain serum samples from the other two patients.

In Winter 1, 42 patients (45.7%) showed a rise in sIgA concentration during the episode of respiratory infection; 31 patients (35.7%) showed a fall and 18 patients (19.6%) showed no change in concentration.

In Winter 2, 33 patients (61%) showed a rise in sIgA concentration, 8 patients (14.8%) showed a fall and 13 patients (24%) showed no change in concentration.

Protein concentrations were measured using a modification of the Lowry technique.

The difference in protein concentration between acute and convalescent specimens was not significant during either study period. (Winter 1 $P > 0.1$ and Winter 2 $P > 0.1$).

In Winter 1, the range of protein concentration was from 5 - 273 mg/dl. The mean protein concentrations for the 85 patients examined were : acute specimens, 56.88 $\pm$ 38.35 mg/dl; convalescent specimens, 53.82 $\pm$ 37.67 mg/dl.

In Winter 2, the range of protein concentration was from 14 - 384 mg/dl. The mean protein concentrations for the 47 patients examined were : acute specimens, 51.82 $\pm$ 59.17 mg/dl; convalescent specimens, 51.22 $\pm$ 53.65 mg/dl.

To facilitate description the patients have been separated into 3 age bands (0-15; 16-29 and $\geq$30 years), and the sIgA levels found in 'acute' and 'convalescent' mucus samples for each age band are shown in figures 10 and 11 for Winter periods 1 and 2.

In Winter 1 the highest percentage of infections is in the expected age band of 0-15 years, followed by the over 30's group and a small percentage of infections in the 16-29 year old group. Winter 2 shows the highest percentage of infection in the over 30's group.

The mean difference in sIgA concentration in each age band is very small. Nevertheless the amount of sIgA shows an increase in each group during both winters.

Expansions of these results are shown in figures 12 to 17 and demonstrate the variation of sIgA between acute and convalescent specimens for each individual patient.

Secretory IgA levels from acute and convalescent specimens of patients over 60 years old in the two winter periods combined are presented in figure 18.

Having established that the difference in sIgA concentration between 'acute' and 'convalescent' specimens was statistically significant, the factors affects sIgA production were examined.

1. sIgA production in patients from whom a respiratory pathogen has been isolated. (Table 30, a) through d)).

In Winter 1, 31 pathogens were isolated from 92 patients: seventeen bacteria (18.5%) and fourteen viruses (15.2%).

Streptococcus viridans and Haemophilus influenzae were the most commonly isolated bacterial respiratory pathogens in Winter 1. Bacteria were isolated equally well from acute or convalescent specimens. Three patients also show infection with Influenza type A. The overall difference in sIgA concentration is approaching significance (P = 0.1/0.05). Twelve out of the seventeen patients (70.6%) showed a difference in sIgA concentration, 7 patients (41.2%) showing a rise and 5 patients (29.4%) showing a fall. Five patients (29.4%) showed no difference in sIgA concentration.

Influenza type A and Rhinoviruses were the most commonly isolated viral respiratory pathogens in Winter 1. In this group the difference in sIgA concentration between specimens is not significant. The viruses tended to be isolated from the acute specimens (85.7%). Twelve out of 14 patients (85.7%) from whom a virus was isolated showed a difference in sIgA concentration, 8 (57.1%) showed a rise, 4 (28.6%) showed a fall and 2 (14.3%) showed no difference in sIgA concentration.

When the above two sets of results are combined to indicate sIgA production when a respiratory pathogen has been isolated, the difference in sIgA concentration is approaching significance. (P = 0.1/0.05).

A wider range of viruses was isolated in Winter 2. The difference in sIgA concentration between specimens is significant. (P = 0.05/0.025). Eight viruses were isolated from 54 patients (14.8%). Forty-one (75.9%) showed a difference in sIgA concentration, 33 (61%) showed a rise and 8 (14.8%) showed a fall. Thirteen patients (24%) showed no change in sIgA concentration.

2. <u>sIgA production with respect to initial sIgA levels</u> (Table 31). Both winters show that if the initial level of sIgA shows a rocket height of $\leq$ 5mm. then the difference in sIgA concentration between acute and convalescent phase specimens is significant. In Winter 1, the range of initial sIgA was 0 - 12mm and 5 patients showed no detectable sIgA in either specimen. In Winter 2, the range of initial sIgA was 2 - 7.5mm and all patients had detectable levels of sIgA.

3. <u>sIgA production with respect to the number of days after the onset of symptoms to collection of the 'acute' specimen</u> (Table 32)

In Winter 1 if the acute specimen is taken 2 - 3 days after the onset of symptoms, the difference in sIgA between the acute and convalescent specimens is significant. Specimens taken at more than 7 days post onset also show a significant difference but care must be taken in interpreting statistical values on such a small number of specimens.

In Winter 2 the results appear to agree. Again a small number of specimens taken at 5 days post onset show a significant difference in sIgA concentration.

4. sIgA production with respect to both the number of days after onset and the height of the initial rocket
(Table 33)

Although the groups tend to be small the figures for both winter periods correspond. The results suggest that specimens with an initial level of sIgA giving a rocket height of 3 - 5mm and taken 2 - 3 days after the onset of symptoms will show a significant difference in sIgA concentration when a convalescent phase specimen is examined about 7 days later.

5. sIgA production with respect to the age of the patient
(Table 34)

In Winter 1, only specimens taken from the under 4 year olds show a significant difference between specimens. In Winter 2, all age groups show a significant difference.

6. Duration of secretory antibody production

During the two winter periods, 14 patients required medical attention on at least two occasions and an acute and convalescent specimen was taken at each episode of respiratory infection. The sIgA concentrations and protein concentrations are shown in figure 19 together with the time interval between respiratory infections. Despite the fact that sIgA and protein levels follow an individual pattern, generally the production of protein follows a similar pattern to that of sIgA and this is confirmed in figures 20 and 21 which are combined graphs for the 14 patients. Both sIgA and protein concentrations fall steadily towards baseline levels over an eight month period but rise again rapidly following re-infection.

7. Dynamics of sIgA production

The accumulation of sIgA and protein in the acute and convalescent specimens is shown in figures 22 and 23 for both Winters. In both periods sIgA accumulation initiated very quickly and the majority of patients had detectable sIgA one day after the onset of symptoms. However, the magnitude of the sIgA response is not great. The protein concentrations varied considerably.

3.2.3 ANALYSIS OF PATIENTS WITH FREQUENT RESPIRATORY INFECTIONS AND/OR ALLERGIES

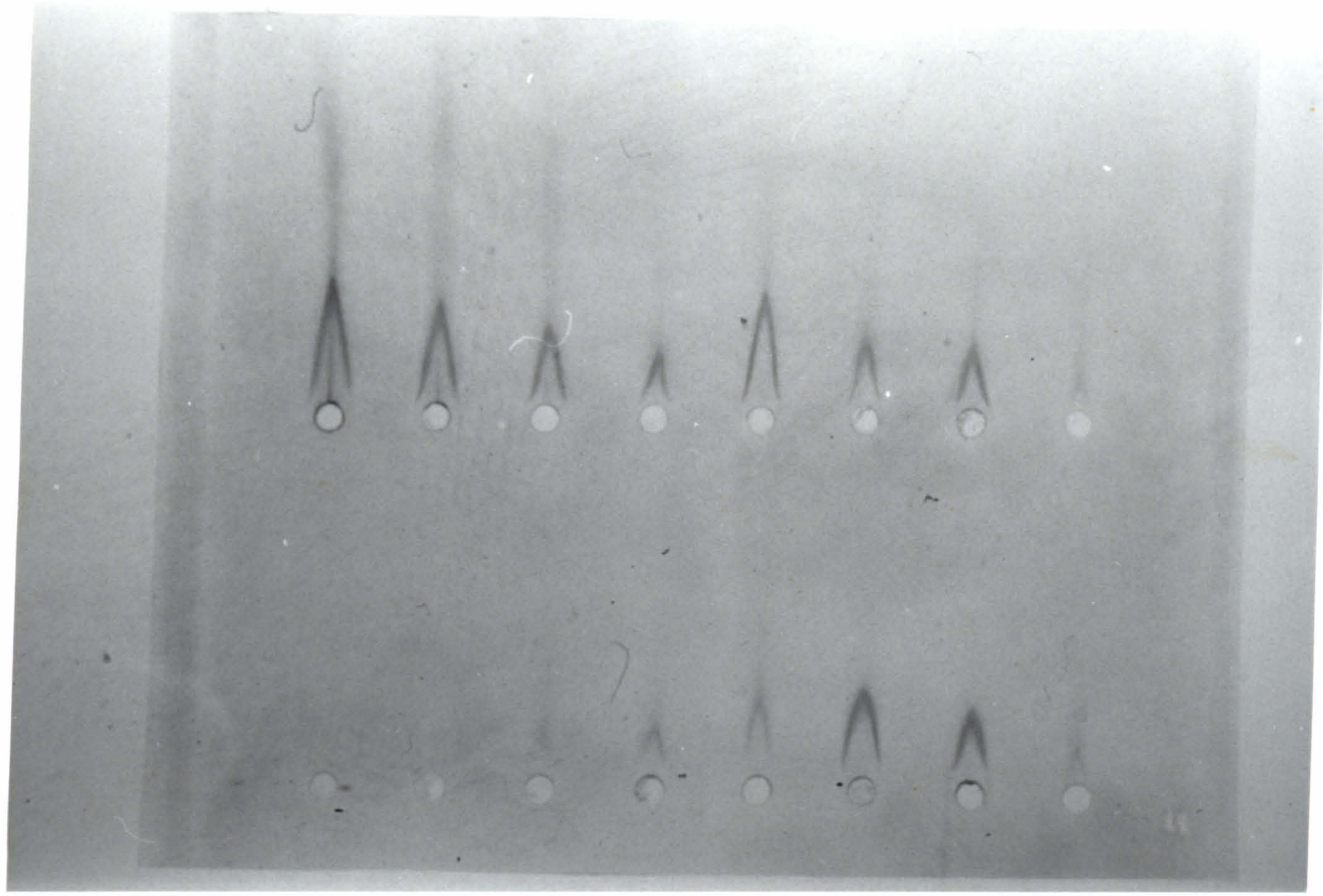
When the sIgA and protein concentrations are examined with regard to whether the levels rise, fall or show no change, the results are shown in Tables 35 and 36. Over the two winter periods the majority of patients showed a rise in sIgA production. Protein concentrations do not show a similar pattern.

When the sIgA and protein concentrations are examined in patients with a history of frequent respiratory infections and/or allergies the results are shown in tables 37 and 38. A graphical comparison is shown in figures 24 and 25. From these results it can be seen that respiratory tract sufferers show a very similar pattern of sIga and protein production to the overall group studied.

ROCKET ELECTROPHORESIS : used to measure sIgA in nasal specimens.

PLATE 1.

1 8



9 16

1. 260
2. 130
3. 65
4. 32.5

Secretory IgA Standards (mg/dl).

5 - 16 : Paired specimens.

KEY FOR TABLE 29 - 30.

A	Acute specimen
C	Convalescent specimen
F	Female
M	Male
N.K.	Not known
N.T.	Not tested
occ.	Occasional
chron.	Chronic
Resp. infs.	Respiratory infections
URTI	Upper respiratory tract infections

Note : In the Pathogen column, the numbers in parenthesis indicate the specimen from which the pathogen was isolated.

Table 29. GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
1.	1270788 1271362	2	M	11 6	94 58	7	NK	Infrequent	
2.	1270789 1271363	27	F	3 4	61 32.5	7	1	Infrequent	
3.	1270790 1271652	31	F	3 6	37 46.5	12	1	Infrequent	
4.	1270972 1271361	9	M	4.5 4.5	17 60	4	1	Infrequent	H.influenzae and pneumococci(2)
5.	1270973 1271531	7	M	6.5 7	38 59	7	NK	Infrequent (has eczema)	Candida sp. (1 and 2)
6.	1270974 1271532	48	M	0 0	25.5 11	7	7	Infrequent	
7.	1270977 1271366	6/12	F	4.5 2	37 23	4	2	Frequent(family) Not allergic	
8.	1271136 1271650	32	M	5 2	34 36	7	1	Infrequent (children have	frequent colds)
9.	1271530 1271981	4	M	5 4	18.5 33.5	9	1	Infrequent	
10.	1271533 1271982	3	M	3 4	28 48.5	8	2	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
11.	1271725 1272123	46	M	2 10	17 46	7	2	Infrequent	Strep.pyogenes (Lancefield A) (1) Candida sp. (2)
12.	1271728 1272122	38	M	0 5	30 ND	7	1	1-2 colds per year	
13.	1271877 1272235	29	F	3 4	5 27.5	7	4	1-2 colds per winter	
14.	1271985 0180057	28	F	4 5	23.5 38.5	7	NK	Infrequent	
15.	1271986 0180056	4	F	3 5	40 68	7	NK	Frequent	
16.	1271987 0180058	30	F	6 10	23 ND	7	7	Infrequent	Rhinovirus
17.	1272124 0180216	33	F	3.5 3	39 18	7	4	Infrequent	
18.	1272126 0180053	3	M	4 2	94 31	7	NK	Frequent	
19.	1272233 0180336	4	M	3 6	50 70	7	NK	Frequent	Haemophilus influenzae (2)
20.	1272236 0180335	24	F	3 3	21.5 28.5	7	NK	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
21.	0180054 0180641	66	M	0 0	39 40	7	4	Infrequent (previous myocardial infarction)	
22.	0180055 0180642	2	M	3 7	23 59	7	2	Infrequent (gets chest inf. + wheeze)	Rhinovirus (1)
23.	0180059 0180639	3	M	4 8	18 50	7	5	Frequent colds (not asthmatic)	
24.	0180214 0180771	30	F	4 0	52.5 18	7	3	Infrequent	
25.	0180215 0180789	57	F	5 8	67 NT	7	7	2 colds/winter chest infs.	
26.	0180337 0180790	66	M	8 5	48.5 77.5	7	5	Chron.Bronchitis. (died-carcinoma of the rectum)	Herpes simplex (2)
27.	0180338 0180788	8/12	M	5 6.5	50 NT	7	3	Infrequent	Rhinovirus + Candida sp. (1)
28.	0180431 0181341	31	M	NT	31 NT	9	NK	Infrequent	
29.	0180432 0181222	30	M	7.5 8	90 44	7	3	Infrequent	
30.	0180637 0181337	8/12	M	9 7.5	NT 85.5	7	2	Frequent.	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
31.	0180772 0181410	54	F	7.5 4	75 57	7	NK	Infrequent	
32.	0180791 0181613	4	F	0 6.5	67 55.5	10	NK	Infrequent	
33.	0181221 0181707	31	F	0 5	24.5 38	7	NK	Infrequent (pregnant)	
34.	0181223 0182024	27	F	3 6	57 36.5	9	3	Infrequent	
35.	0181338 0181848	28	M	7 8.5	80.5 70	7	3	Infrequent	
36.	0181340 0181706	22	M	8 6	45 39.5	6	1	Infrequent	
37.	0181612 0182140	2	F	8 5	44 48.5	7	3	Frequent	Candida sp. (1)
38.	0181614 0182137	60	F	5 10	58.5 75	7	6	Infrequent	Candida sp. (2)
39.	0181705 0280011	40	F	6 6	27.5 30.5	6	5	Infrequent	
40.	0182138 0280404	3	F	4 6	53 92	7	7	Frequent	H.influenzae (2)

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
41.	0182139 0280405	1½	F	5 8	88 136	7	2	4-5 colds per year	
42.	0182141 0280406	24	F	5.5 7	28.5 67	7	7	Infrequent	
43.	0280009 0280723	32	F	11 8.5	167 39	8	4	1 cold/chest per winter	Strep.viridans, Candida sp. (1)
44.	0280010 0280609	9/12	F	5 5	75 55	7	2	Infrequent	Strep.viridans, Candida sp. (2)
45.	0280012 0280611	30	F	5.5 8.5	58.5 65	7	4	Frequent	Influenza type A H.Influenzae (1)
46.	0280013 0280610	4	M	8 8	85 72	7	NK	Frequent	H.influenzae (2)
47.	0280014 0280613	65	M	0 8	30 102	7	1	1-2 colds/ bronchitis per winter	Parainfluenza type 3 (1)
48.	0280165 0280979	55	F	9.5 5	104 32	9	5	Infrequent	
49.	0280166 0280612	2	M	6 3	77.5 36	7	5	Infrequent	
50.	0280167 0280726	22	F	5 8.5	65 106	7	4	1-2 colds per year becoming bronchitis (diabetic on insulin)	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	No. of Days: after onset	History of Resp. Infs.	Pathogen (specimen)
51.	0280169 0280725	23	F	0 5	33 26.5	6	4	Infrequent	
52.	0280170 0280724	27	F	6 10	92.5 55	7	2	Infrequent	
53.	0280181 0280757	3½	F	7 4	93.5 59	7	4	Infrequent	Candida sp. (1) H.Influenzae (2)
54.	0280182 0280759	5	F	3 3	53 42.5	6	3	Infrequent	Strep.viridans (1) Candida sp. (2)
55.	0280183 0280758	9	F	6 6	64 66	7	7	Infrequent	
56.	0280400 0280982	4	M	6 6	78 45	7	2	Frequent upper resp.tract infections	H.Influenzae (1)
57.	0280401 0280977	8	M	6 11	52 186	7	1	Frequent	Influenza type A (1) H.Influenzae (2)
58.	0280402 0280983	21	M	8 5	36 54	7	2	Infrequent	
59.	0280403 0280975	34	F	7.5 5	40.5 27	7	5	Infrequent	
60.	0280478 0280981	17	F	6 10	40 92.5	7	2	1 cold per winter	Influenza type A (1)

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
61.	0280481 0281064	16	M	6 4.5	33 17	6	2	Infrequent	Strep.viridans (1)
62.	0280482 0280976	8	M	12 7	158 32	6	4	Infrequent	
63.	0280490 0281066	52	F	5 0	84 44	7	3	Acute bronchitis (Heavy smoker)	
64.	0280491 0281063	26	F	6 5	33 40	7	3	Infrequent	
65.	0280721 0281210	1	F	0 6	48.5 78	6	4	Frequent	Candida sp. (1)
66.	0280722 0281328	30	F	5 4	29 20	7	2	Infrequent (several abortions)	H.influenzae (1)
67.	0280754 0281328	30	M	5 3	72.5 27.5	6	1	Frequent	
68.	0280755 0281061	18	F	0 0	19 9	4	2	Infrequent (sister is a diabetic)	
69.	0280756 0281493	36	F	7 5	82 67	7	4	Infrequent	
70.	0280980 0281492	35	M	5 7	71.5 85.5	4	3	Frequent	H.influenzae (1)

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
71.	0281062 0281620	65	M	7.5 5	68.5 18.5	7	1	Chronic bronchitis	Herpes simplex(1)
72.	0281065 0282058	6	M	5 5	52 51	8	3	Frequent colds and chest infs	Influenza type A (1) Strep. viridans (2)
73.	0281207 0281930	6	M	3 6	46.5 47	7	2	Frequent	Influenza type A(1)
74.	0281208 0281811	25	F	5.5 7.5	27.5 46.5	6	4	Infrequent	
75.	0281209 0281810	30	F	0 0	17.5 19.5	6	1	Allergic rhinitis	Influenza type A(1)
76.	0281211 0282149	47	M	9 3	78.5 51	9	3	Infrequent	
77.	0281324 0282057	28	M	6 4.5	55.5 47	7	2	Infrequent	
78.	0281325 0282150	25	M	6.5 4.5	116 52.5	7	1	Infrequent	
79.	0281326 0281809	22	F	5 7.5	16 34	5	2	Infrequent	
80	0281621 0282273	4	M	6 8	64 66.5	7	1	Frequent URTI, chest and ear infs.	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	No. of Days: after onset	History of Resp. Infs.	Pathogen (specimen)
81.	0281622 0282274	1½	F	5 5	32 122	7	3	Infrequent	
82.	0282054 0380148	5	M	4 4	103 28	7	4	Infrequent	
83.	0282055 0380149	6/12	F	4.5 0	76.5 26	10	NK	Infrequent	
84.	0380150 0380591	47	M	0 2	36 28.5	4	NK	Infrequent	
85.	0380301 0380949	20	F	T 5	41.5 31.5	6	NK	Infrequent	
86.	0380592 0381075	5	F	4 4	36 24	7	NK	Infrequent	
87.	0380950 0381539	39	M	0 4.5	26 33.5	6	NK	Infrequent	
88.	0381429 0382072	1	M	0 4.5	46 75	7	NK	Infrequent	Strep.viridans. (2)
89.	0381430 0382073	2	M	2 0	87 30	7	NK	2-3 colds per winter (brother of above)	Influenza type A (1)
90.	0381538 0382126	2	M	3 9.5	37 147	6	NK	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 1	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
91.	0382127 0382544	2	F	11 13	273 259	8	NK	Infrequent	
92.	0480047 0480490	34	M	0 0	27 45.5	4	NK	Infrequent	
Winter 2									
1.	1181430 1182158	6/12	M	5 5	39.5 46.5	7	3	Very frequent colds and ear infs.	Echovirus type 22 (1&2)
2.	1181777 1182350	5	M	7 5.5	371 76	5	4	Not frequent but severe. (mother has SLE)	
3.	1181778 1182717	2/12	M	6 4	68 31	8	1	Infrequent	
4.	1181779 1182716	27	F	6 7	49 57	8	3	Infrequent	
5.	1181780 1182600	33	M	6 6	55 61	7	1	Infrequent (ecc. septic tonsillitis)	
6.	1181825 1182719	7	F	3.5 5	28.5 54	7	2	Infrequent	
7.	1182154 1182939	16	M	7 10	270 152	7	3	Frequent colds & septic tonsillitis.	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 2	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
8.	1182155 1183142	16	M	5 8	55 59	7	1	Infrequent	
9.	1182156 1183338	8	M	3 6	19.5 27.5	9	4	Infrequent(has eczema)	Parainfluenza type 1 (1)
10.	1182157 1280415	31	F	7 8	ND ND	4	4	Infrequent	
11.	1182351 1280297	1	M	6 8	33.5 53	12	5	Infrequent	
12.	1182431 1280414	21	F	5 6	39.5 31	13	1	Infrequent	
13.	1182432 1280302	5	M	5 5	43.5 23	12	15	Infrequent	
14.	1182598 1183340	1	M	2 4	39 54	7	2	Frequent colds	
15.	1182599 1183341	5	F	4 8	23 84.5	7	NK	Frequent colds and chest infections. Asthmatic(on Intal)	
16.	1182601 1183337	24	F	6 6	43 28	7	2	Infrequent	
17.	1182718 1280295	24	F	2 3	14.5 21	11	2	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 2	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	No. of Days: after onset	History of Resp. Infs.	Pathogen (specimen)
18.	1182720 1280022	5	M	5 3.5	44 31	8	2	Frequent(family same.7 week baby sister:cot death)	
19.	1182938 1280298	2	M	5 8	32 384	8	1	Frequent colds and chest infs. Asthmatic(+Eczema)	
20.	1182940 1280299	6	M	4 8	35.5 96	8	14	Infrequent	Parainfluenza type 3 (1)
21.	1182941 1280301	23	F	5 6.5	31 70.5	8	2	Infrequent	Influenza type A (1)
22.	1183141 1280413	26	F	3 4	23.5 21	8	2	Infrequent	
23.	1183143 1280604	5	F	5 4	59 29	10	1	Has eczema and is allergic (Brother has asthma)	
24.	1183144 1280536	7	M	6.5 7	70.5 29	9	1	Frequent (probable asthmatic)	
25.	1183140 1280416	6	F	5.5 NT	54	8	1	Infrequent	Parainfluenza type 3 (1)
26.	1183344 1280300	65	F	6 7	59.5 45.5	6	5	Infrequent(freq. tonsillitis. Mother:diabetic)	Parainfluenza type 1 (1)
27.	1183231 1280789	16	M	5.5 6.5	42.5 78.5	9	2	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 2	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	after onset	History of Resp. Infs.	Pathogen (specimen)
28.	1183232 1280540	4	M	4 5	26 45	8	2	Infrequent	
29.	1183233 1280539	28	M	4 5	27.5 24	8	2	Infrequent	
30.	1183343 1280601	13	M	6 6	23 18.5	8	2	2-3 colds per winter	
31.	1183339 1280603	82	F	7.5 5.5	104 55	8	7	Infrequent	
32.	1183345 1280607	26	M	5 5	23.5 27.5	9	1	Infrequent	
33.	1280021 1280787	5	F	6.5 7.5	36 39	7	2	Frequent	
34.	1280023 1270790	10/12	M	5.5 8	81.5 68	7	5	Infrequent	
35.	1280024 1280788	20	F	5 6	26.5 24	8	5	Frequent URTI's	
36.	1280296 1281031	14	F	4.5 7.5	29.5 38	7	0	Infrequent	
37.	1280304 1281029	24	F	4 5	16.5 20.5	7	2	Infrequent	

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 2	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	No. of Days: after onset	History of Resp. Infs.	Pathogen (specimen)
38.	1280417 1281077	8	F	5.5 7.5	33 51.5	7	6	Frequent URTI. and tonsillitis.	
39.	1182942 1280020	26	M	4 5.5	41.5 29.5	5	2	Frequent URTI. and tonsillitis.	
40.	1182943 1280303	4	F	4 7	30.5 100	8	2	Infrequent	
41.	1280537 1281383	46	F	2 3	14 22	7	5	Infrequent	
42.	1280538 1281382	9	M	3.5 4	16 41	7	3	Infrequent	
43.	1280541 1281429	35	M	7 3	78 21	7	4	Infrequent	
44.	1280602 1281428	22	F	4 4	24.5 28.5	7	3	Infrequent	
45.	1280605 1281030	47	M	3 6	80 36	5	3	1-2colds/ chest infs.per winter.	
46.	1280606 1281078	28	F	4 5	33.5 19.5	5	4	Infrequent	
47.	1280781 1281568	7	F	3 4.5	35 34.5	7	2	Infrequent	Influenza type A (1)

Table 29 (continued) GENERAL PRACTICE STUDY : ADDITIONAL INFORMATION

Winter 2	Specimen No	Age	Sex	sIgA (Rocket ht.mm)	Protein (mg/dl)	No. of Days: between specs.	No. of Days: after onset	History of Resp. Infs.	Pathogen (specimen)
48.	1280782 1281567	2	M	3.5 3.5	24.5 20.5	7	2	Infrequent	
49.	1280783 1281079	25	M	5.5 4	32.5 32	5	1	Infrequent	Influenza type A (1)
50.	1280784 1281569	23	M	3 3	44 20.5	7	5	Infrequent	
51.	1281032 1281809	1	M	3 4	78.5 50	7	4	Frequent URTI.	
52.	1281073 1282029	11	M	3 2	20 22	8	3	Infrequent	
53.	1281074 1281810	11	M	4 5	>100 75.5	7	3	Frequent URTI.	
54.	1281075 1281982	29	F	4 4	26 47	8	4	Infrequent	

Table 30 sIgA production in patients from whom a respiratory pathogen has been isolated

a) Winter 1 Bacterial Isolates

Patient	A	C	Difference	Bacterium isolated
4	4.5	4.5	0	Haemophilus influenzae(c)
11	2	10	+8	Streptococcus pyogenes(A)
19	3	6	+3	H. influenzae (c)
40	4	6	+2	H. influenzae (c)
43	11	8.5	-2.5	Step. viridans (A)
44	5	5	0	Strep. viridans(A)
45	5.5	8.5	+3	H. influenzae* (A)
46	8	8	0	H. influenzae (c)
53	5	4	-1	H. influenzae (c)
54	3	0	-3	Strep. viridans (A)
56	6	6	0	H. influenzae (A)
61	6	4.5	-1.5	Strep. viridans (A)
66	5	4	-1	H. influenzae (A)
70	5	7	+2	H. influenzae (A)
72	5	5	0	Strep. viridans* (c)
88	0	4.5	+4.5	Strep. viridans (c)
57	6	11	+5	H. influenzae* (c)

N = 17 d = 1.09

SD = 2.9

SE = 0.7

t = 1.56 degrees of freedom = 16

P = 0.1/0.05

Difference is approaching significance

*Influenza type A was also isolated from these patients

Table 30 (continued).

b) Winter 1 Viral isolates

Patient	A	C	Difference	Virus isolated
8	5	2	-3	Rhinovirus (c)
16	6	10	+4	Rhinovirus (A)
22	3	7	+4	Rhinovirus (A)
26	8	5	-3	Herpes simplex (c)
27	5	6.5	+1.5	Rhinovirus (A)
45	5.5	8.5	+3	Influenza type A*(A)
47	0	8	+8	Parainfluenza type 3(A)
57	6	11	+5	Influenza type A*(A)
60	8	9.5	+1.5	Influenza type A(A)
71	7.5	5	-2.5	Herpes simplex (A)
72	5	5	0	Influenza type A*(A)
73	3	6	+3	Influenza type A(A)
75	9	3	-6	Influenza type A (A)
89	2	0	-2	Influenza type A (A)

N = 14 d = 0.96

SD = 3.87

SE = 1.03

t = 0.932 degrees of freedom = 13

P = 0.2/0.15

Difference is not significant

*Pathogenic bacteria also isolated from these patients

Table 30 (continued).

c) Winter 1 Respiratory Pathogen (combination of tables a and b)

N = 28

d = 0.86

SD = 3.378

SE = 0.638

t = 1.35 degrees of freedom = 27

P = 0.1/0.05

Difference is approaching significance.

Table 30 (continued).

d) Winter 2 Viral isolates

Patient	Rocket HT (mm) A	C	difference	Virus isolated
1	5	5	0	Echovirus type 22
9	3	6	+3	Parainfluenza type 1
20	4	8	+4	Parainfluenza type 3
21	5	6.5	+1.5	Influenza type A
25	5.5	7	+1.5	Parainfluenza type 3
26	6	7	+1	Parainfluenza type 1
47	3	4.5	+1.5	Influenza type A
49	5.5	4	-1.5	Influenza type A

N = 8

d = 1.375

SD = 1.685

SE = 0.596

t = 2.31 degrees of freedom = 7

P = 0.05/0.025

Difference is significant

NB All specimens were isolated from the first specimen

Table 31 sIgA production with respect to initial sIgA levels

Winter 1

Rocket Ht (mm)	N	d	SD	SE	t	d of f	P
≤ 3.5	30	2.82	2.74	0.5	5.64	29	0.0005
4	1.0	-0.6	2.66	0.32	1.8	9	0.05
5	19	0.74	2.51	0.58	1.28	18	>0.1
6	13	0.15	2.99	0.86	0.17	12	>0.1
7	7	-0.21	2.16	0.88	0.24	6	>0.1
8	5	-1.9	1.95	0.97	1.96	4	>0.1
≥ 9	6						NT

Winter 2

Rocket Ht (mm)	N	d	SD	SE	t	d of f	P
≤ 3.5	13	1	1.17	0.33	3.03	12	0.01/0.005
4	12	1.71	1.42	0.43	3.89	11	0.01/0.005
5	15	0.83	1.48	0.4	2.08	14	0.05/0.025
6	10	0.15	1.29	0.43	0.35	9	>0.1
7	4	0.13	2.32	1.34	0.1	3	>0.1

Table 32 sIgA production with respect to the number of days after the onset of symptoms that the'acute' specimen was taken.

Winter 1

No. of days after onset	N	d	SD	SE	t	d of f	P
1	14	0.68	3.3	0.88	0.77	13	> 0.1
2	17	0.94	2.87	0.695	1.35	16	0.1
3	13	-1.04	2.88	0.22	4.73	12	< 0.0005
4	13	0.62	3.25	0.90	0.69	12	> 0.1
5	6	-1.5	3.07	1.25	1.2	5	> 0.1
6	1						NT
⩾ 7	6	1.75	1.6	0.71	2.46	5	0.05/0.025

Winter 2

No. of days after onset	N	d	SD	SE	T	d of f	P
1	11	0.45	1.72	0.57	0.79	10	> 0.1
2	17	0.76	1.03	0.25	3.04	16	0.05/0.0025
3	8	0.94	1.43	0.54	1.74	7	0.1/0.05
4	6	0.75	1.47	0.66	1.14	5	> 0.1
5	5	1.3	0.97	0.49	2.65	4	0.05/0.025
⩾ 6	4	1	2.58	1.49	0.67	3	> 0.1

Table 33 sIgA production with respect to the number of days after onset and the height of the initial rocket

Winter 1

Rocket Ht (mm) / No. of days	0 - 2mm	3 - 5mm	6 - 8mm	9mm
1	N=4 P = 0.1	N=7 P = 0.1	N=5 P = 0.1	-
	N=11 P = 0.1			
2	N=3 P = 0.1	N=8 P = 0.1	N=6 P = 0.1	N=1
	N=11 P = 0.1/0.05			
3	N=1 ND	N=8 P = 0.1	N=5 P = 0.1	-
	N=9 P = 0.05			
4	N=3 P = 0.1	N=5 P = 0.1	N=4 P = 0.1	N=2
	N=8 P = 0.1/0.05			
5	-	N=5 P = 0.01/0.005	N=9 P = 0.1	N=1

Table 33 (continued).

Winter 2

	0 - 2mm	3 -5mm	6 - 8mm	9mm
1	-	N=7 P=0.1	N=3 P=0.1	-
2	N=2	N=13 P=0.125/ 0.01	N=2	-
3	-	N=7 P=0.1/ 0.05	N=1	-
4	-	N=14 P=0.1	N=3 P=0.1	-
5	N=1	N=3 P=0.1		-
6	-	N=3 P=0.1		-

Table 34 sIgA production with respect to the age of the patient

Winter 1

Age(years)	N	d	SD	SE	d of f	t	P
≤ 4	28	0.804	3.18	0.602	27	1.33	0.1/0.05
5-12	10	0.35	2.91	0.91	9	0.38	>0.1
13-21	5	0.9	3.47	1.55	4	0.58	>0.1
> 21	44	0.46	3.06	0.48	40	0.96	>0.1
≥ 60	5	1.25	4.36	1.95	4	0.64	>0.1

Winter 2

Age (years)							
< 4	11	1	1.72	0.54	10	1.85	0.05/0.025
5-12	16	1.03	1.64	0.41	15	2.51	0.125/0.01
13-21	6	1.5	1.22	0.55	5	2.73	0.025/0.0125
> 21	19	0.55	1.29	0.3	18	1.83	0.05/0.025
> 60	2	-	-	-	-	-	NT

Table 35 Summary of sIgA levels (Rocket heights)

Winter 1

Age range	Number	sIgA concentration Rise	Fall	No change
0-15years	38	16	11	11
16-29years	21	13	6	2
≥ 30years	33	12	13	7
Total	92	41 (44.6%)	30 (32.6%)	20 (ND=1) (21.8%)

Mean base-line level of sIgA = 4.6 mm

Winter 2

Age range				
0-15years	29	19	5	5
16-29years	18	12	1	5
≥ 30years	7	4	2	1
Total	54	25 (61%)	8 (14.8%)	11 (24%)

Mean base-line level of sIgA = 4.8 mm

Table 36 Summary of Protein levels (mg/dl)

Winter 1

Age range	Number	Protein concentration Rise	Fall	No change
0-15 years	38	15	12	9
16-29 years	21	8	6	7
≥ 30 years	33	7	13	9
Total	92	30 (32.6%)	31 (33.7%)	25 (ND=6) (27.2%)

Winter 2

Age range				
0-15 years	29	10	9	8
16-29 years	18	3	3	12
≥ 30 years	7	0	4	3
Total	54	13 (24%)	16 (29.6%)	23 (42.6%)

Table 37 Analysis of sIgA levels (rocket heights) in patients with frequent respiratory infections and/or allergies

Winter 1

Age range	Number	Rise	Fall	No change
0-15 years	15/38	8	4	3
16-29 years	1/21	1	0	0
≥ 30 years	7/33	2	4	1
Total	23/92	11	8	4
		(47.8%)	(34.8%)	(17.4%)

Mean base-line level of sIgA = 4.8

Winter 2

Age range				
0-15 years	12/29	5	2	5
16-29 years	3/18	3	0	0
≥ 30 years	0/7	0	0	0
Total	15/34	8	2	5
		(53.3%)	(13.3%)	(33.3%)

Mean base-line level of sIgA = 4.7

Table 38 Analysis of protein levels (mg/dl) in patients with frequent respiratory infections and/or allergies

Winter 1

Age range	Number	Rise	Fall	No change	
0-15 years	15/38	6	3	5	
16-29 years	1/21	1	0	0	
≥ 30 years	7/33	2	3	2	
Total	23/92	9	6	7	(ND = 1)
		(39%)	(26%)	(30.4%)	

Winter 2

Age range				
0-15 years	11/29	4	5	2
16-29 years	4/18	1	1	2
≥ 30 years	0/7	0	0	0
Total	15/54	5	6	4
		(33.3%)	(40%)	(26.7%)

Figure 10. sIgA Levels. (Winter 1).

— Mean rocket height
□ Virus isolated
▲ Bacteria isolated
■ Both Virus and Bacteria isolated
○ No pathogen isolated

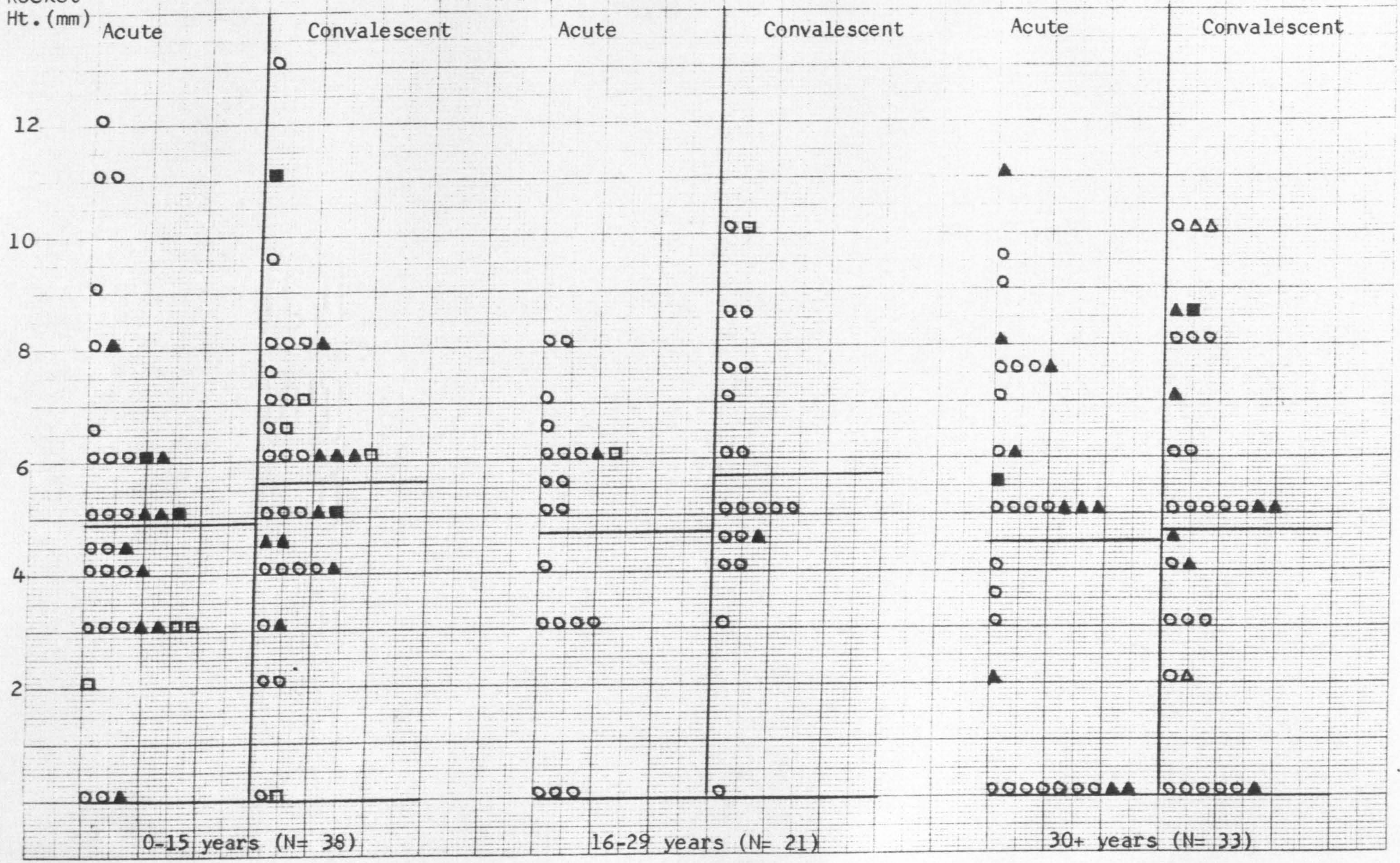


Figure 11. sIgA Levels. (Winter 2).

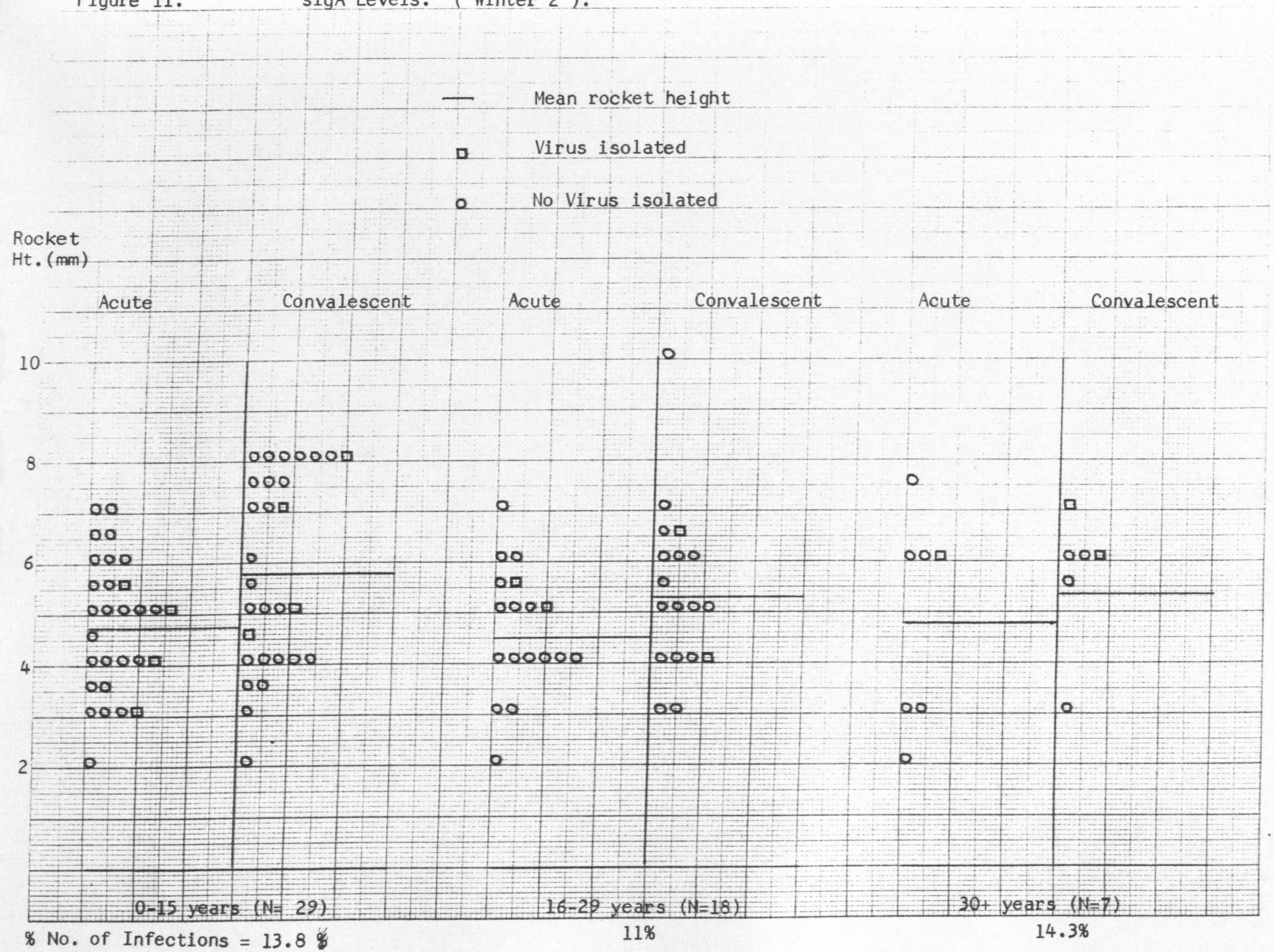


Figure 12. Paired sIgA Levels. (Age range 0-15 years).Winter 1.

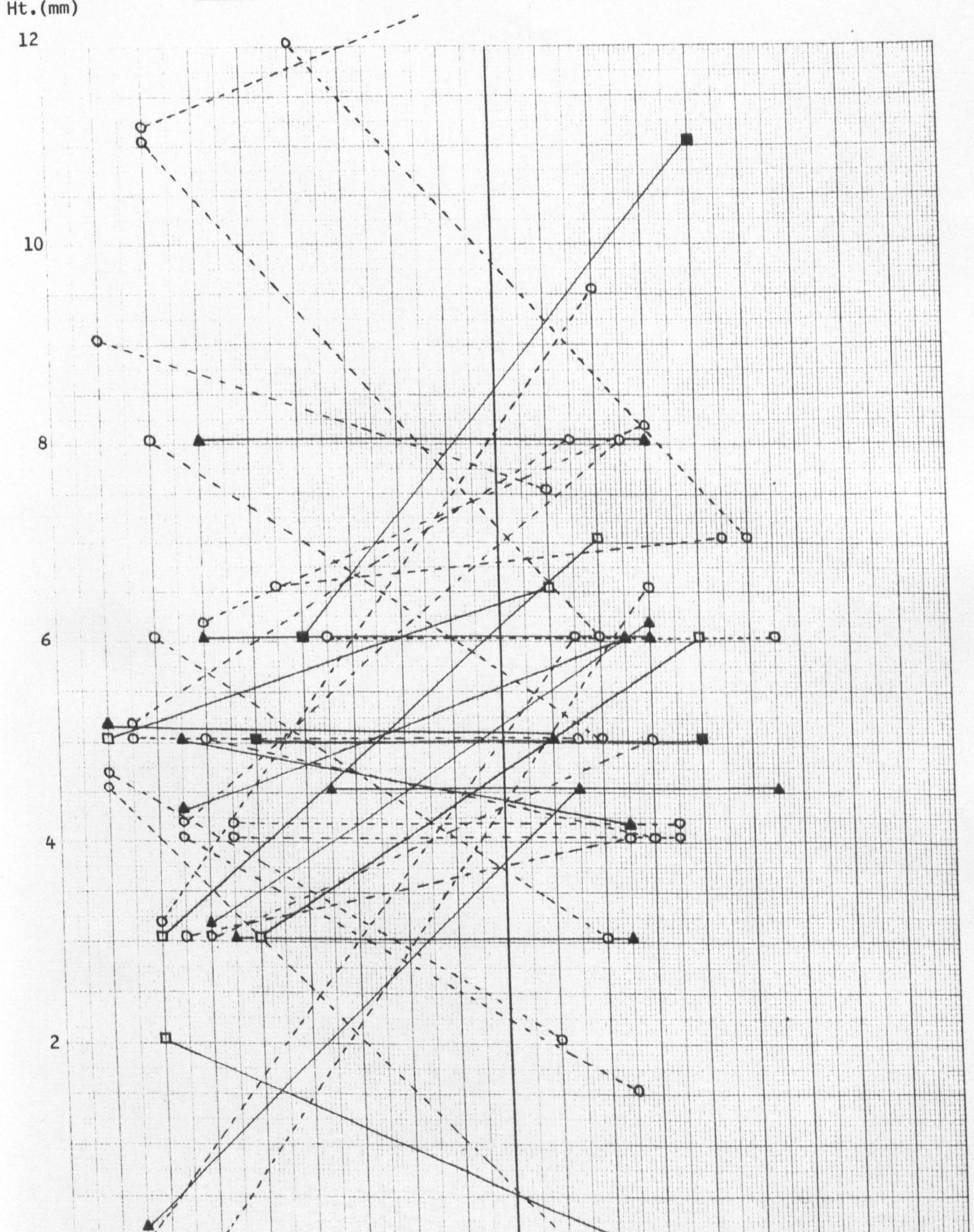


Figure 13. Paired sIgA Levels. (Age range 16-29 years). Winter 1.

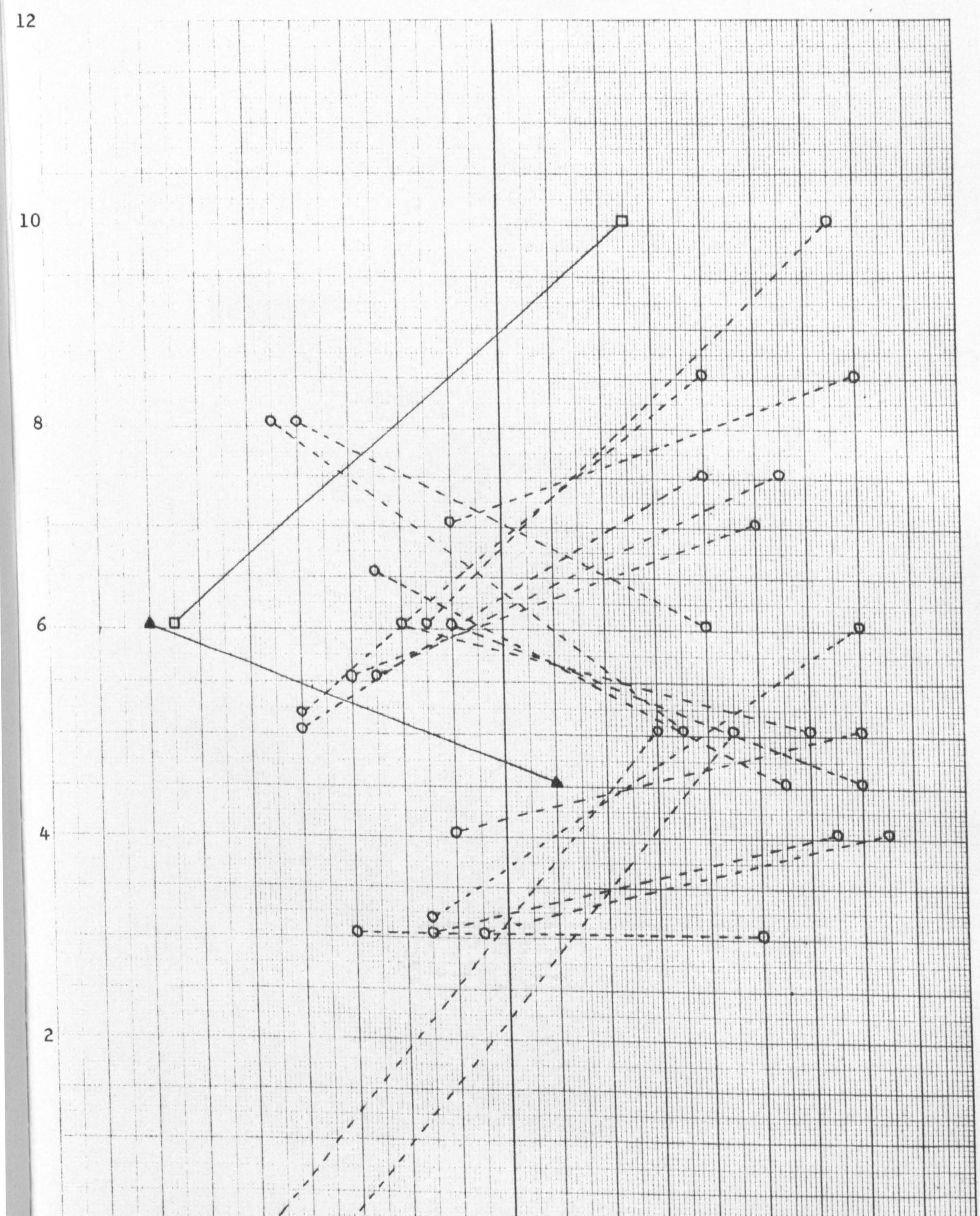


Figure 14. Paired sIgA Levels. (Age range 30+ years). Winter 1.

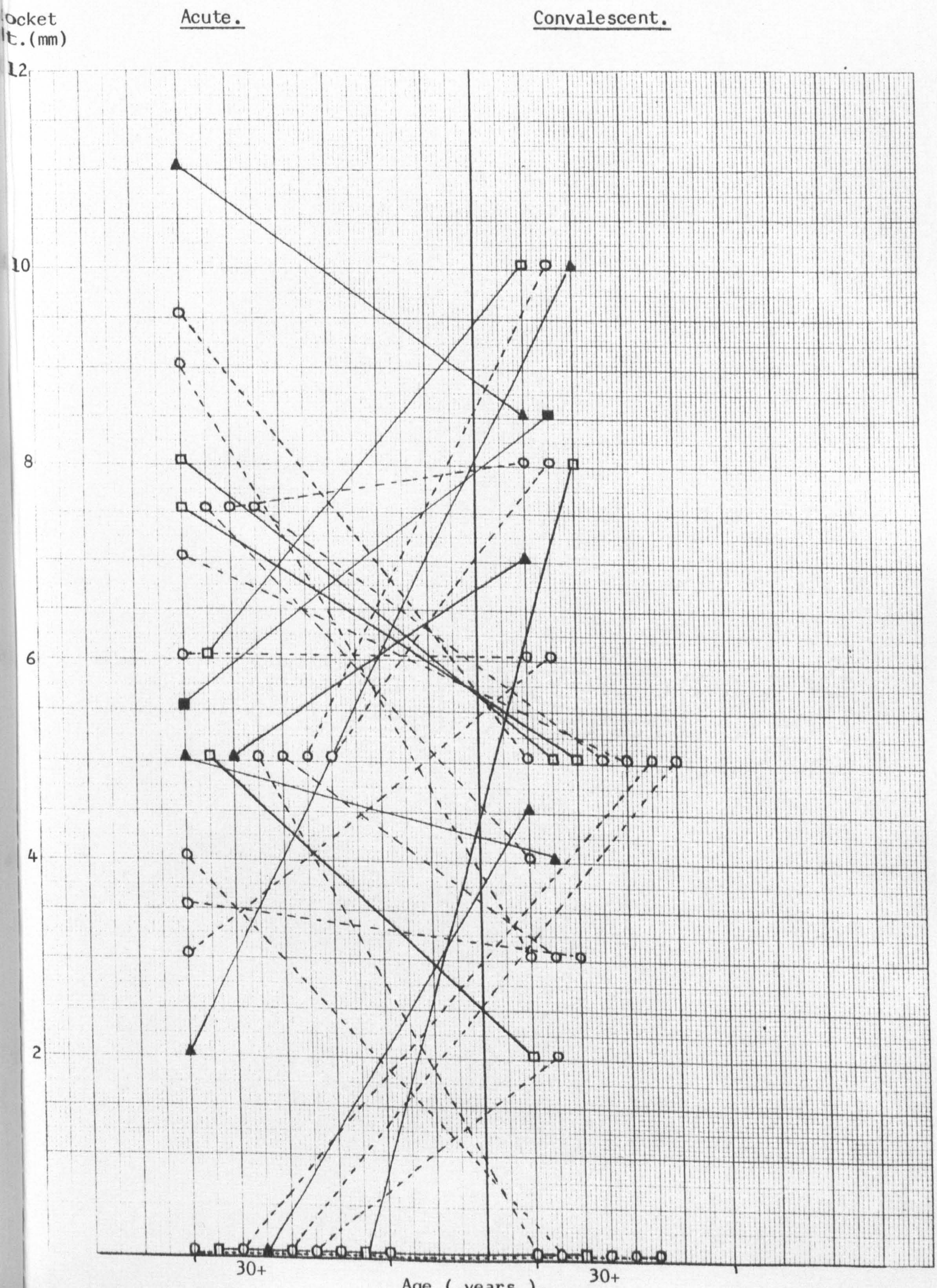


Figure 15. Paired sIgA Levels. (Age range 0-15 years). Winter 2.

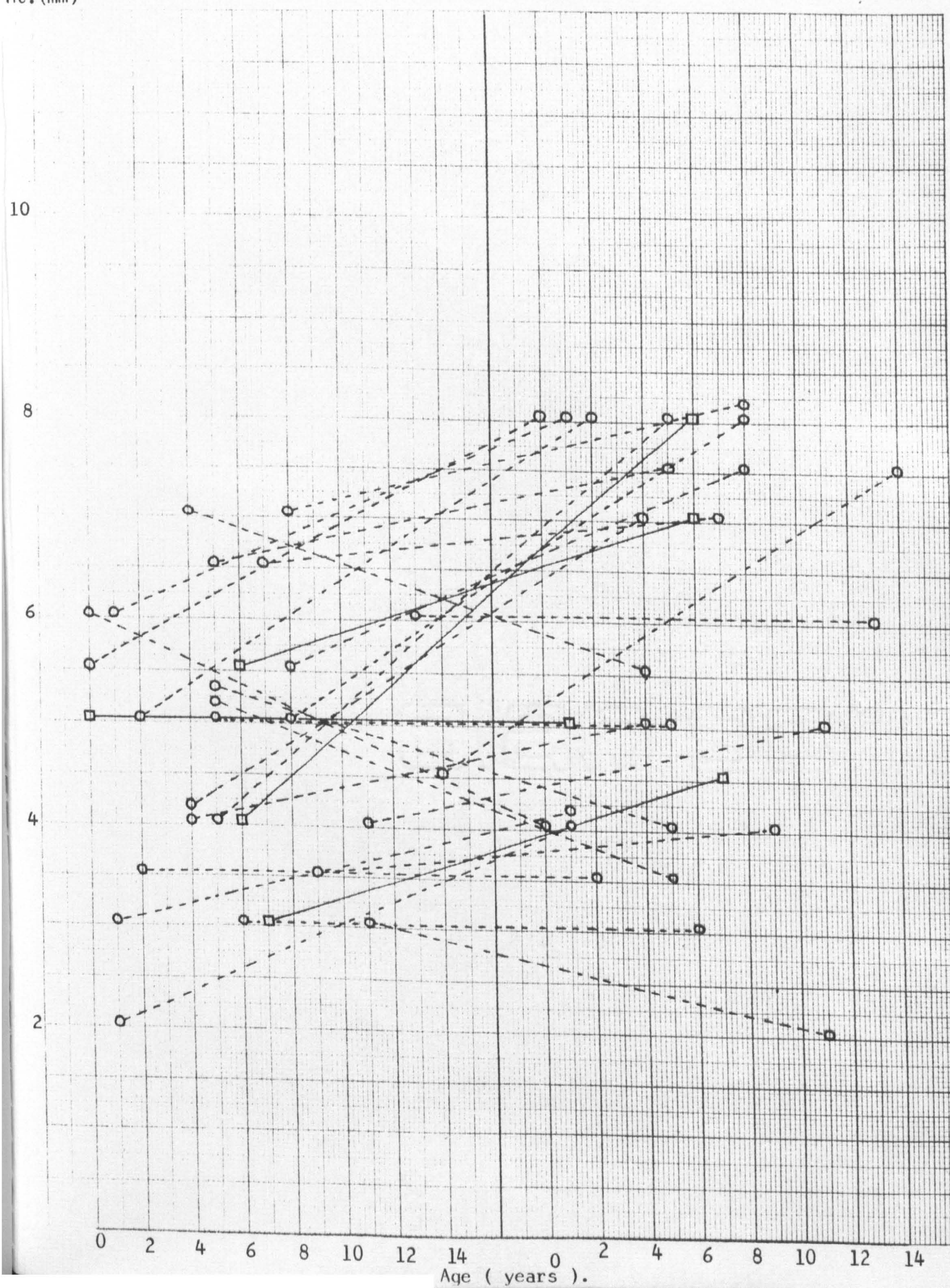


Figure 16. Paired sIgA Levels. (Age range 16-29 years). Winter 2.

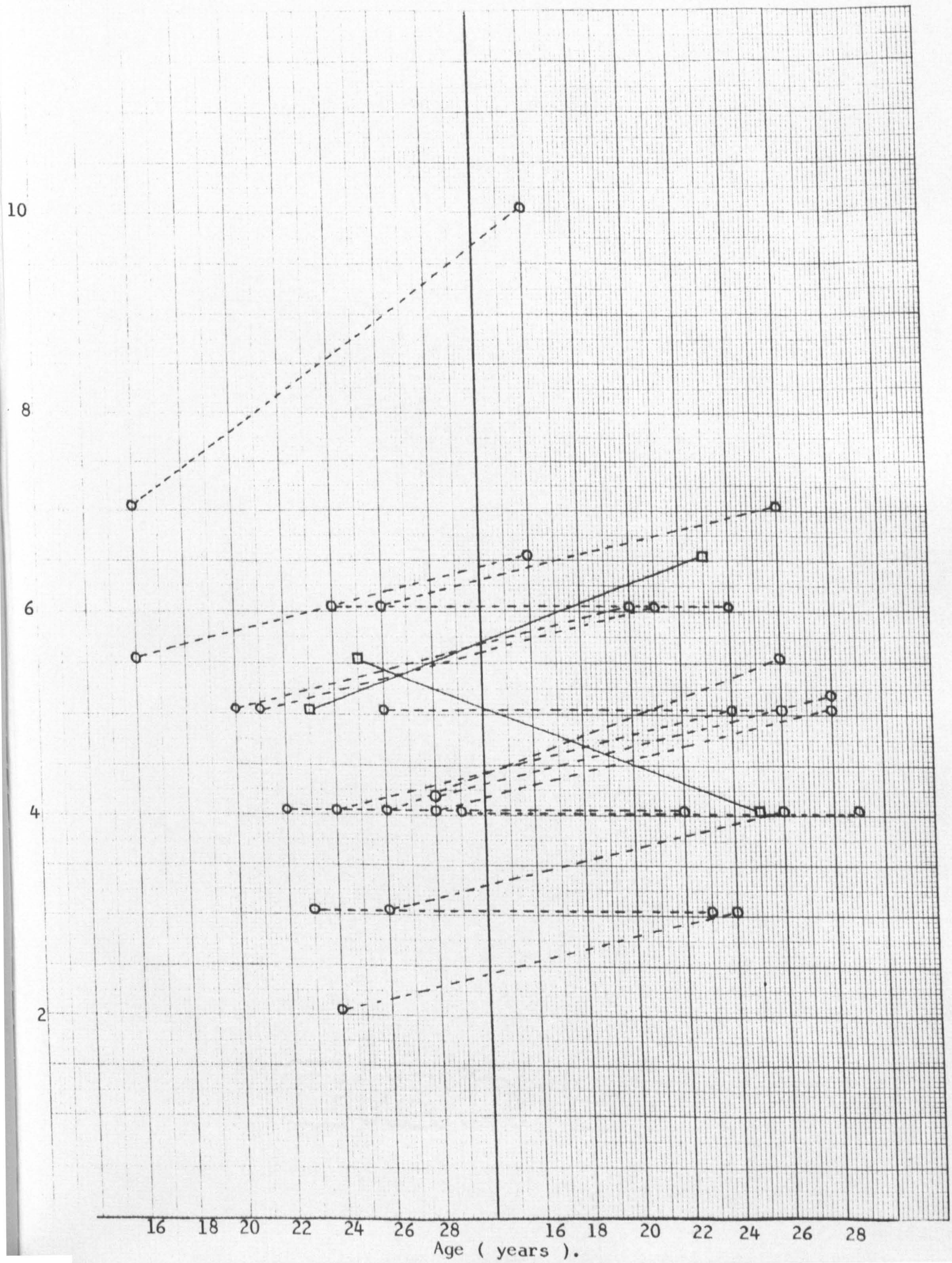


Figure 17. Paired sIgA Levels. (Age range 30+ years). Winter 2.

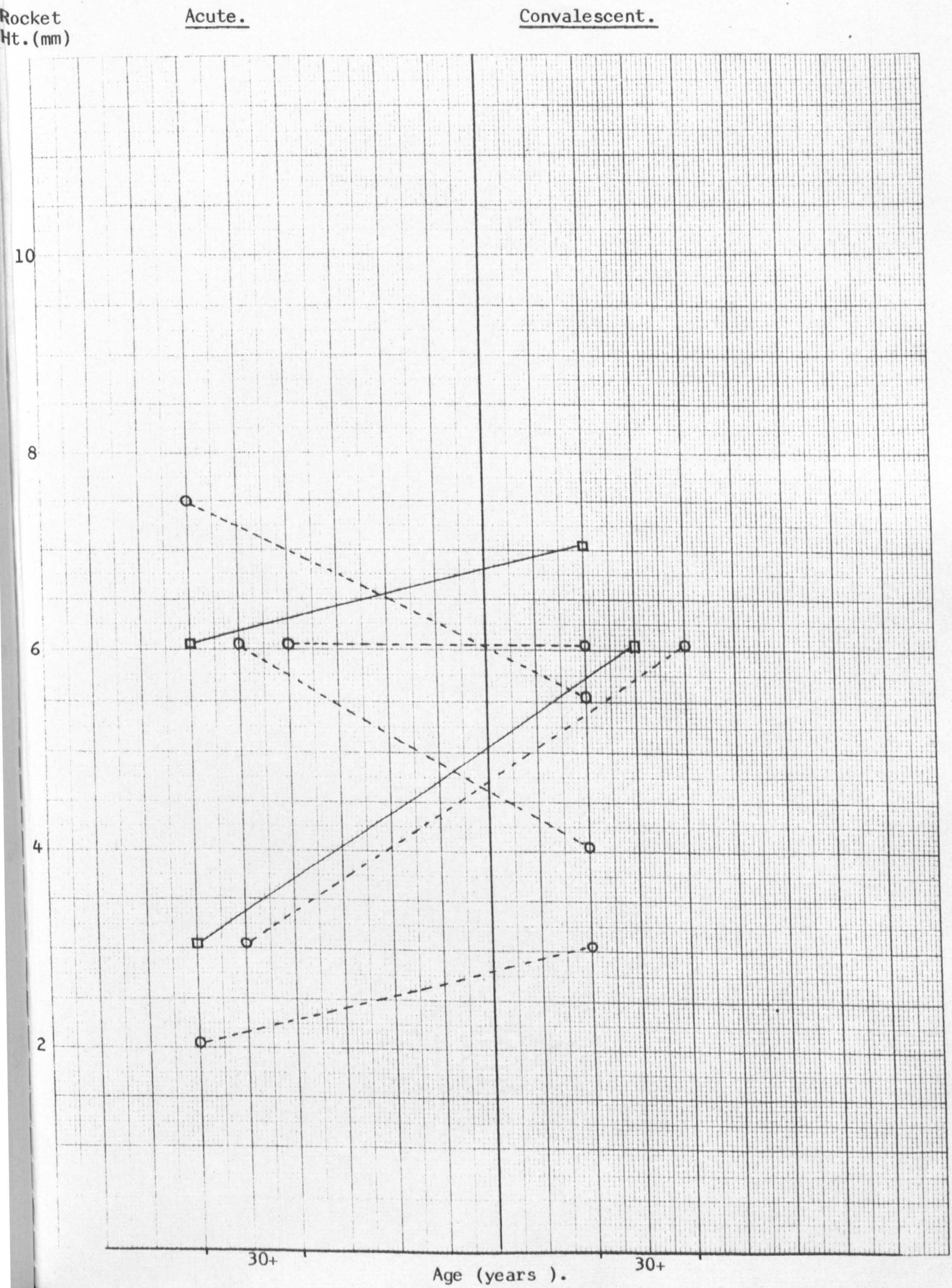
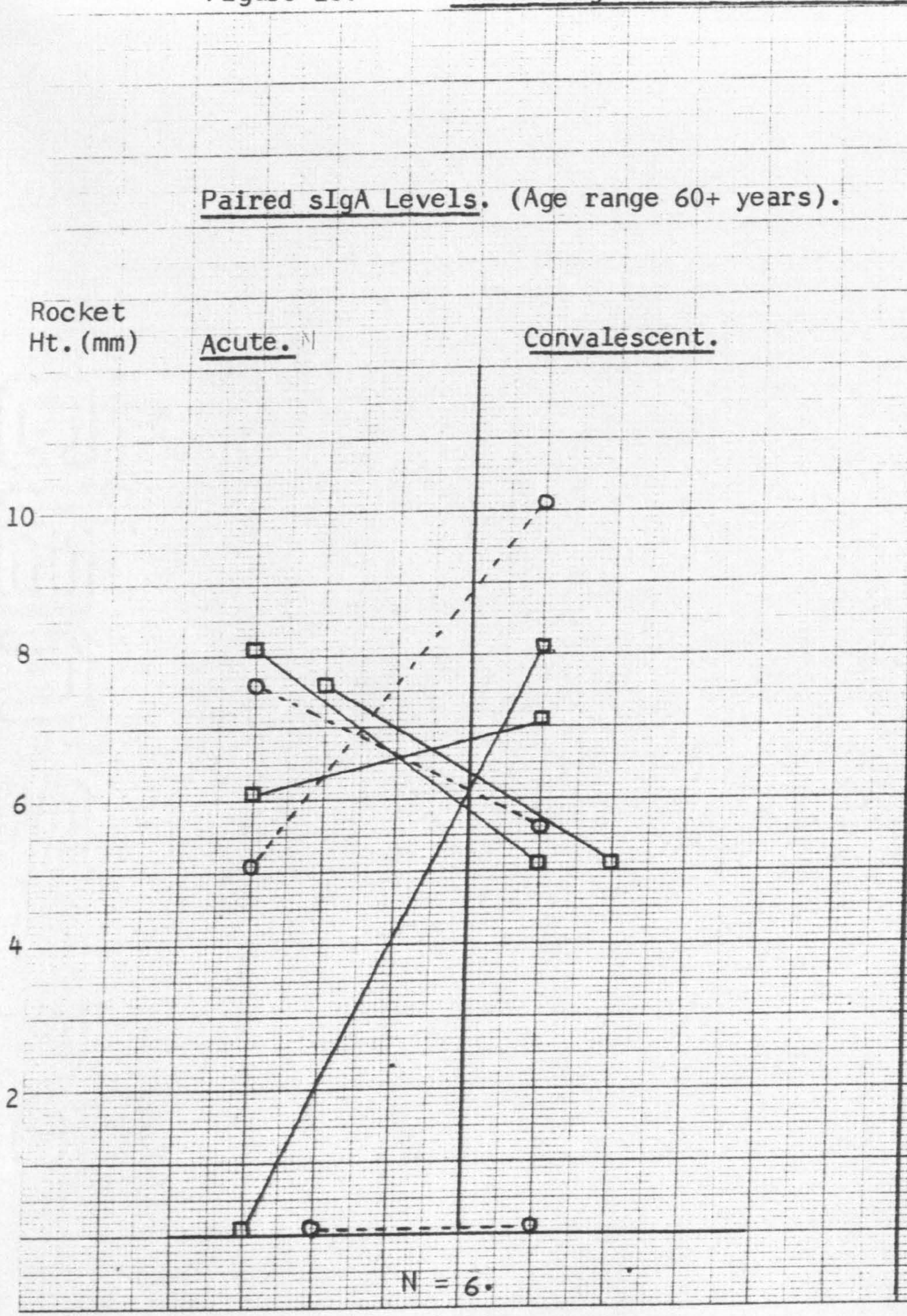


Figure 18. Paired sIgA and Protein Levels. (Age range 60+ years). Winters 1 & 2 combined.



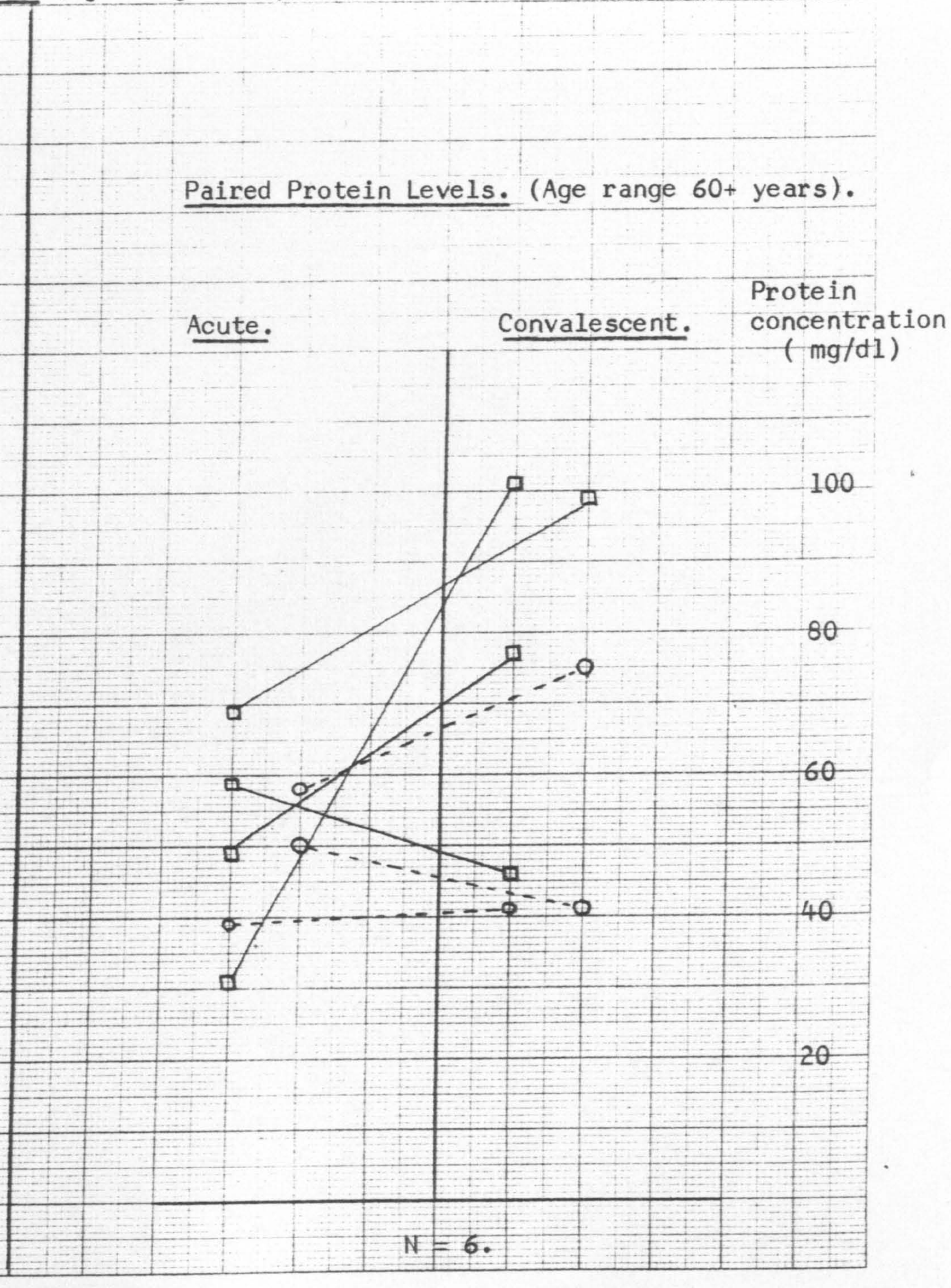


Figure 19. Duration of Antibody Production.

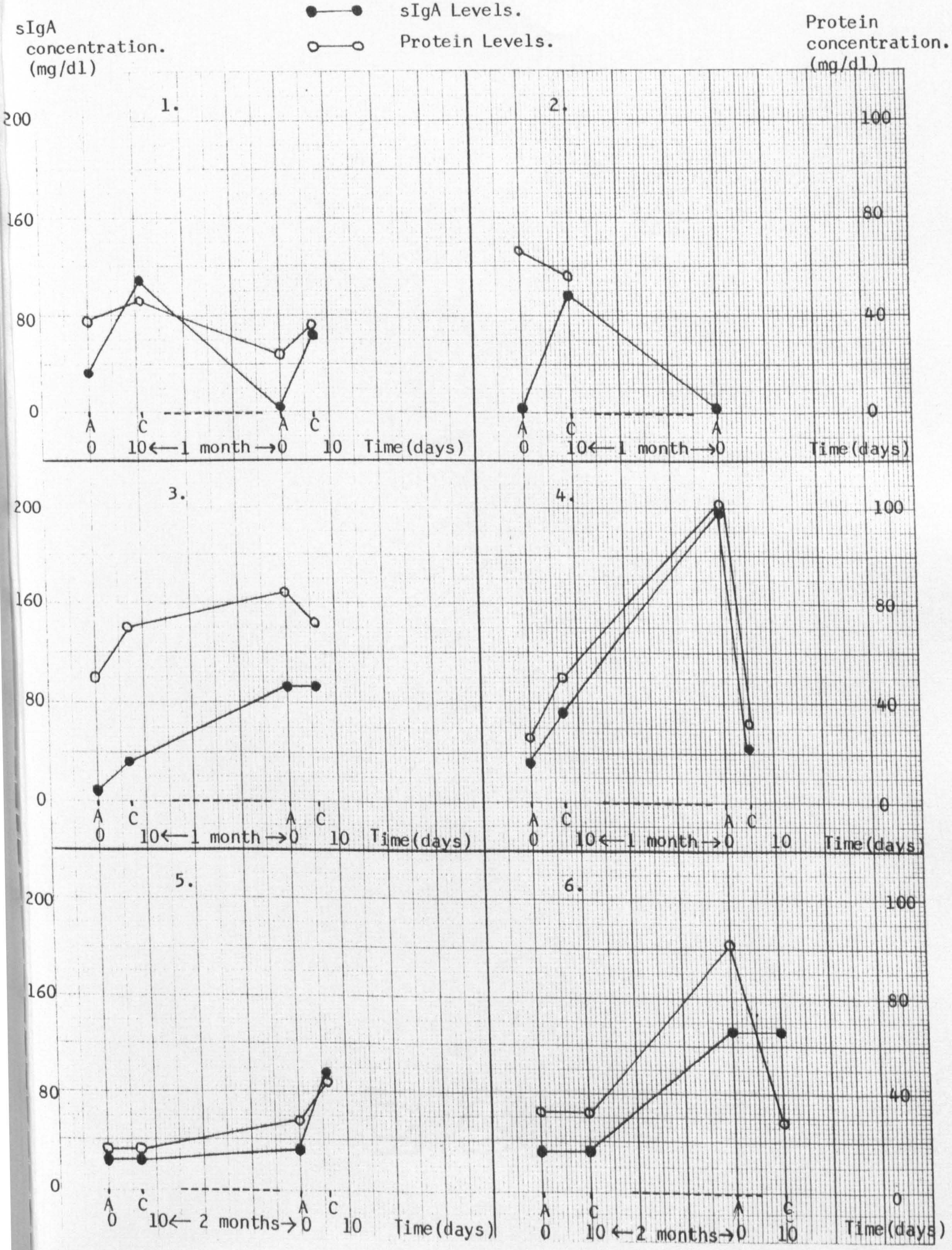


Figure 19 (continued). Duration of Antibody Production.

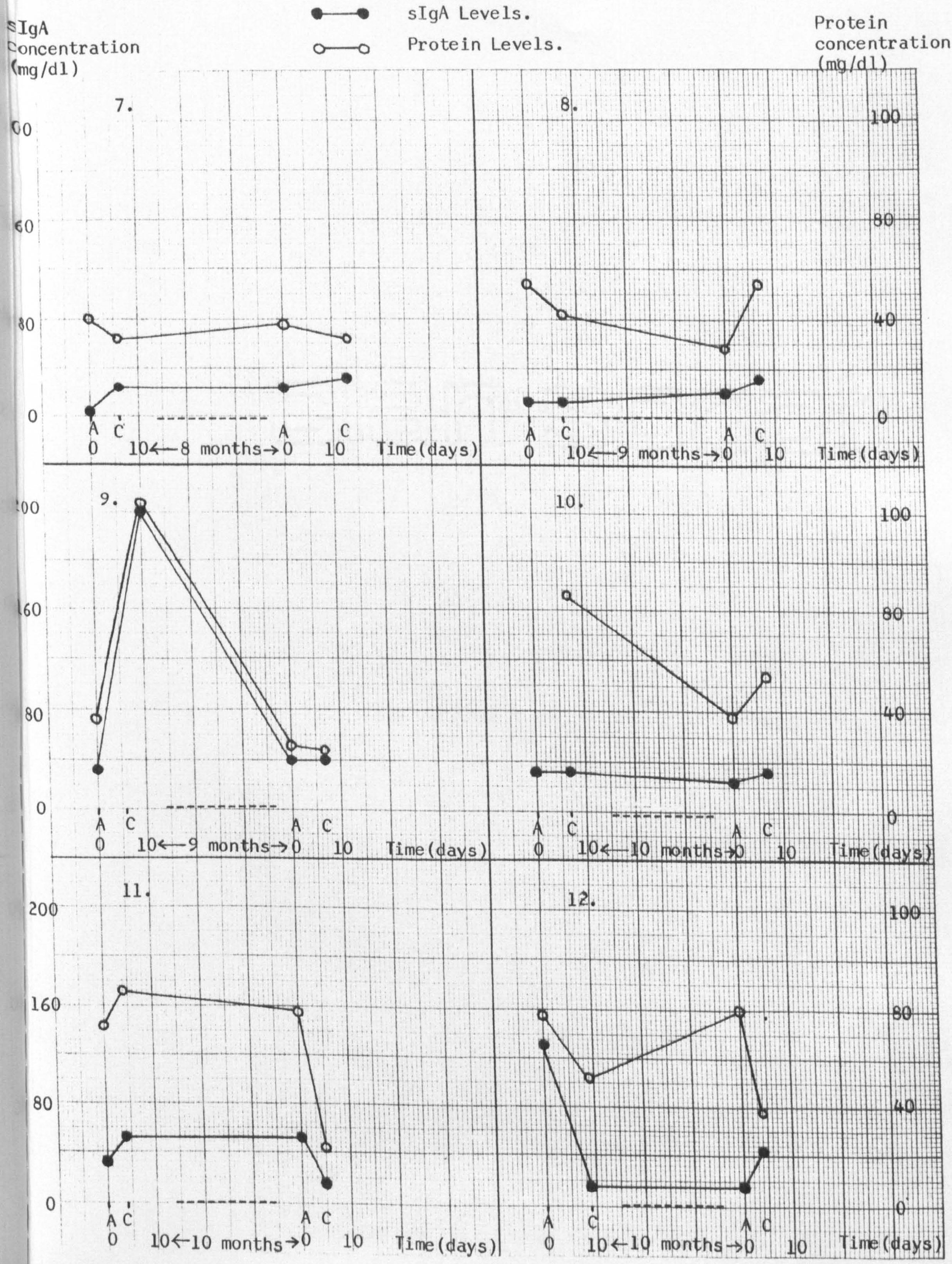
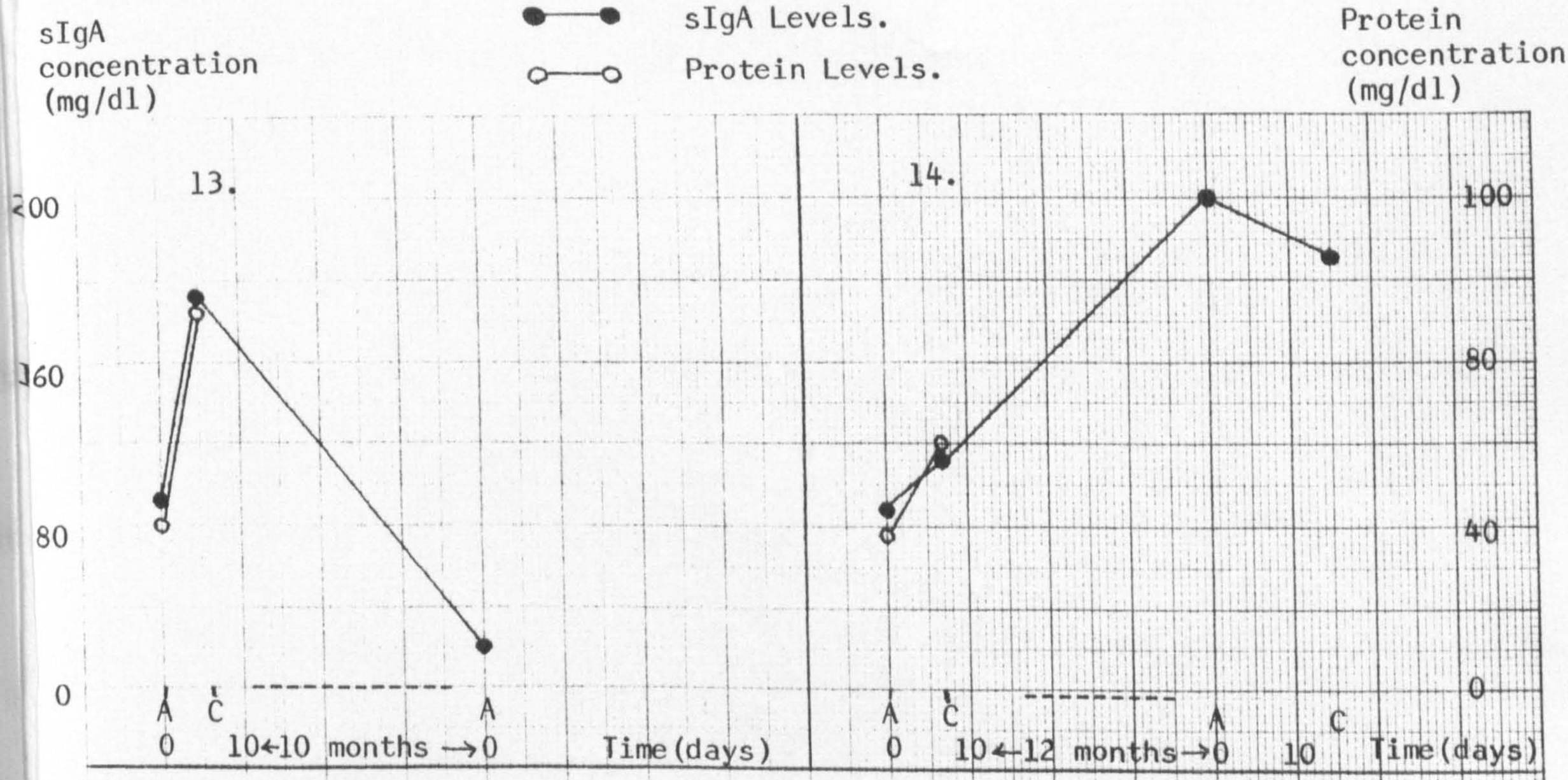


Figure 19 (continued). Duration of Antibody Production.



Note :

These sIgA levels are measured in mg/dl. because these specimens were retested together at a later stage of the project when a sIgA standard had become available.

Figure 20. Duration of Antibody Production. (N = 14)

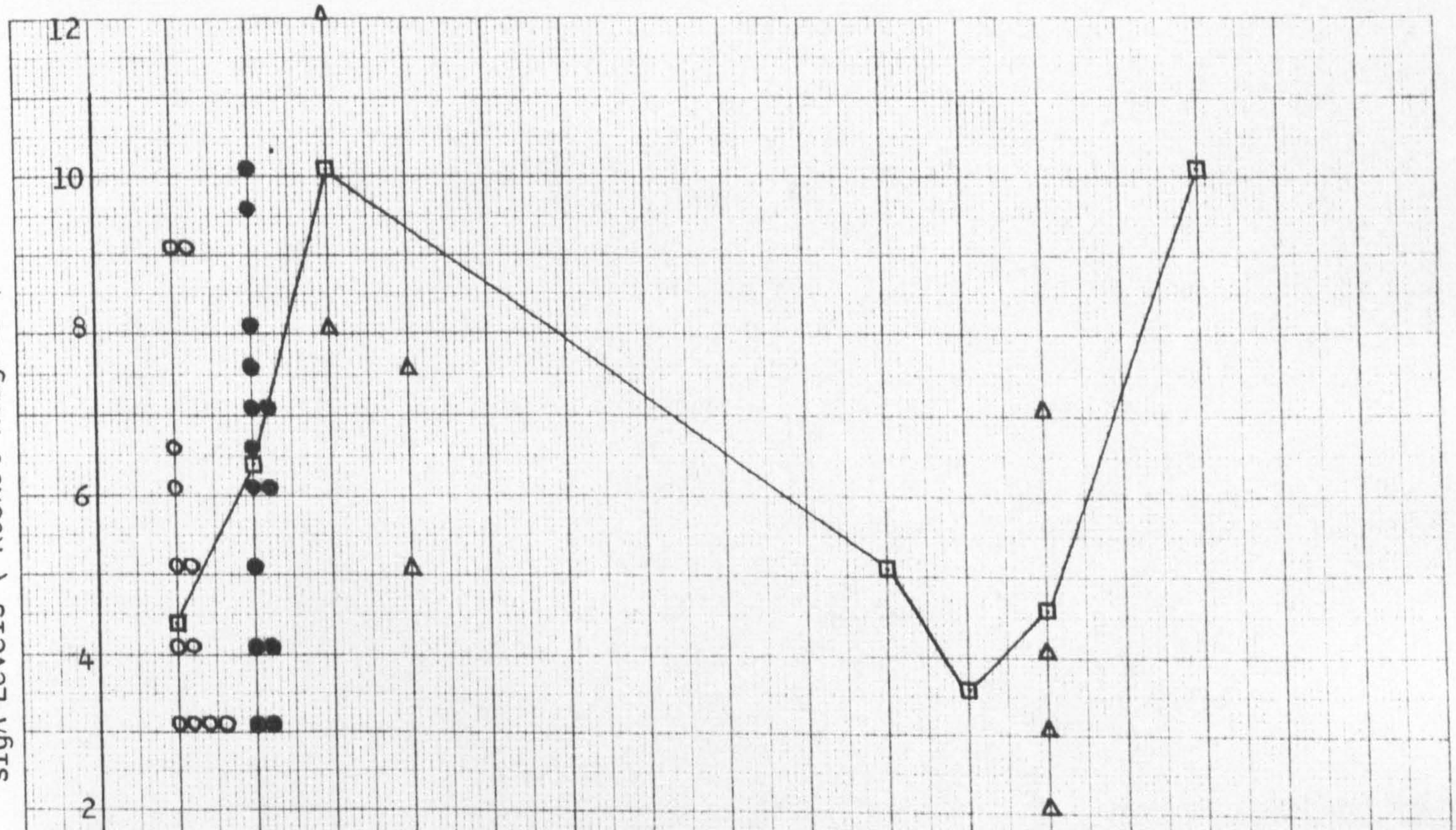


Figure 21. Duration of Protein Production. (N = 14)

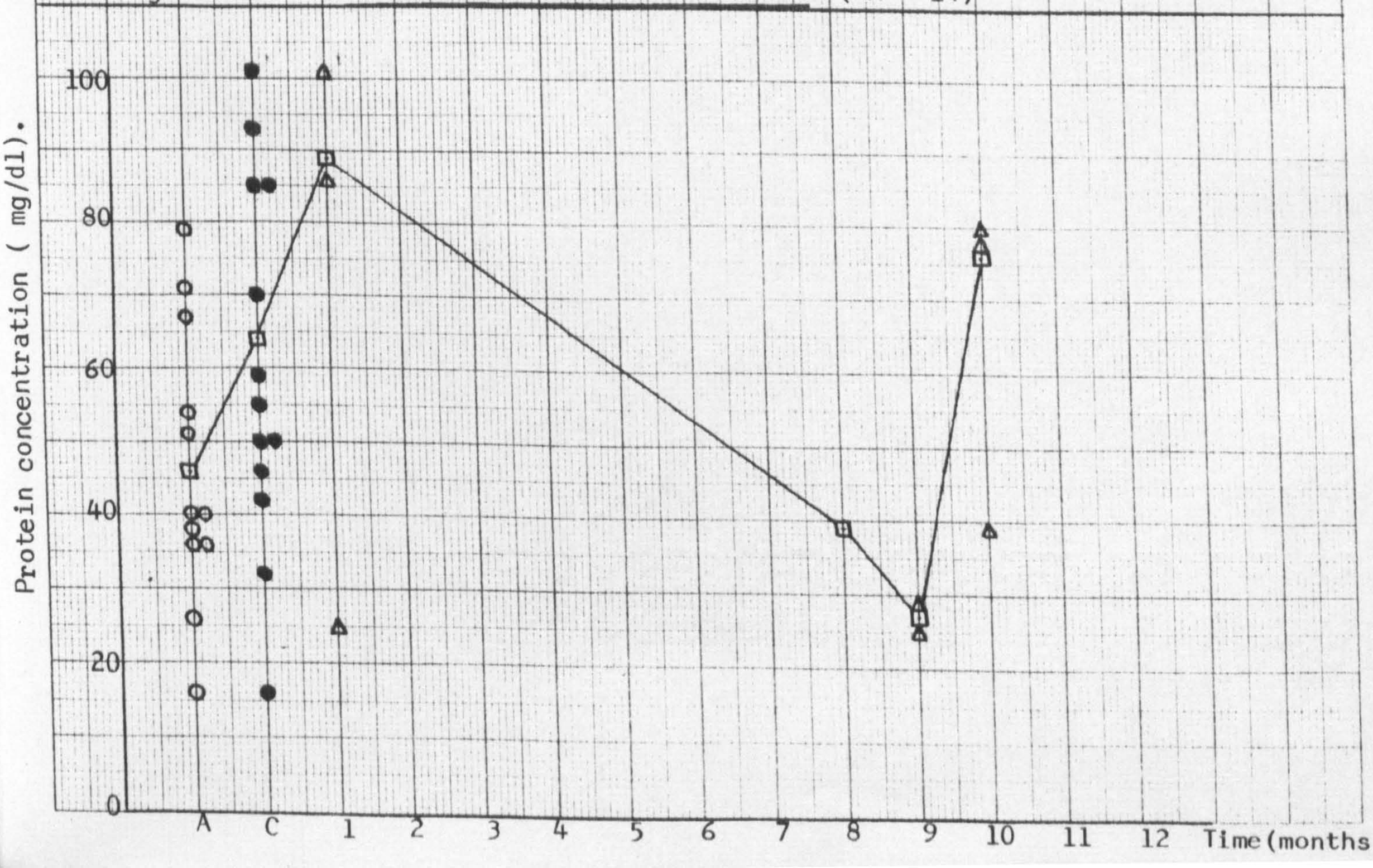


Figure 22. sIgA Levels with respect to the Number of Days After the Onset of Symptoms.

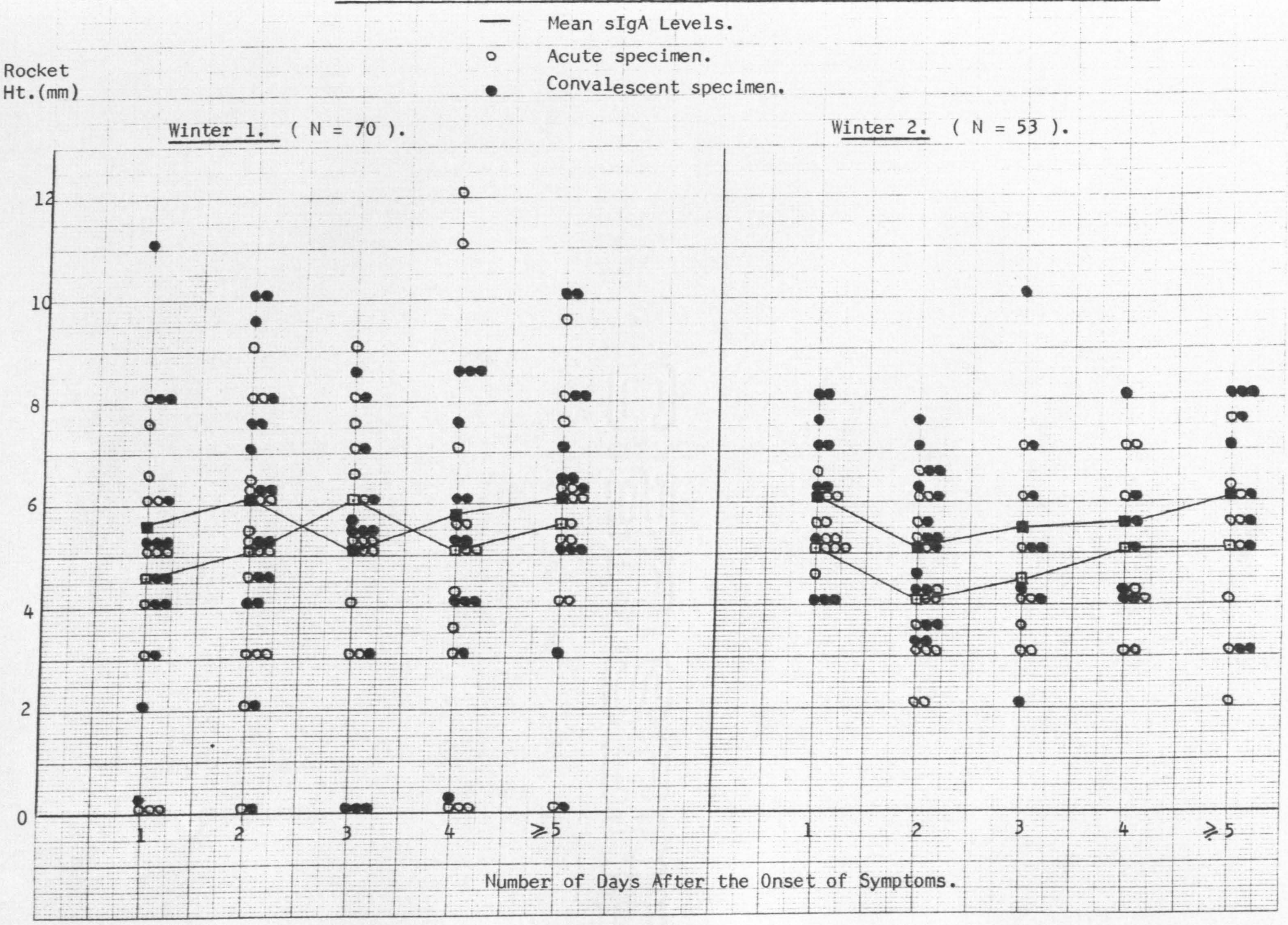


Figure 23. Protein Levels with respect to the Number of Days After the Onset of Symptoms.

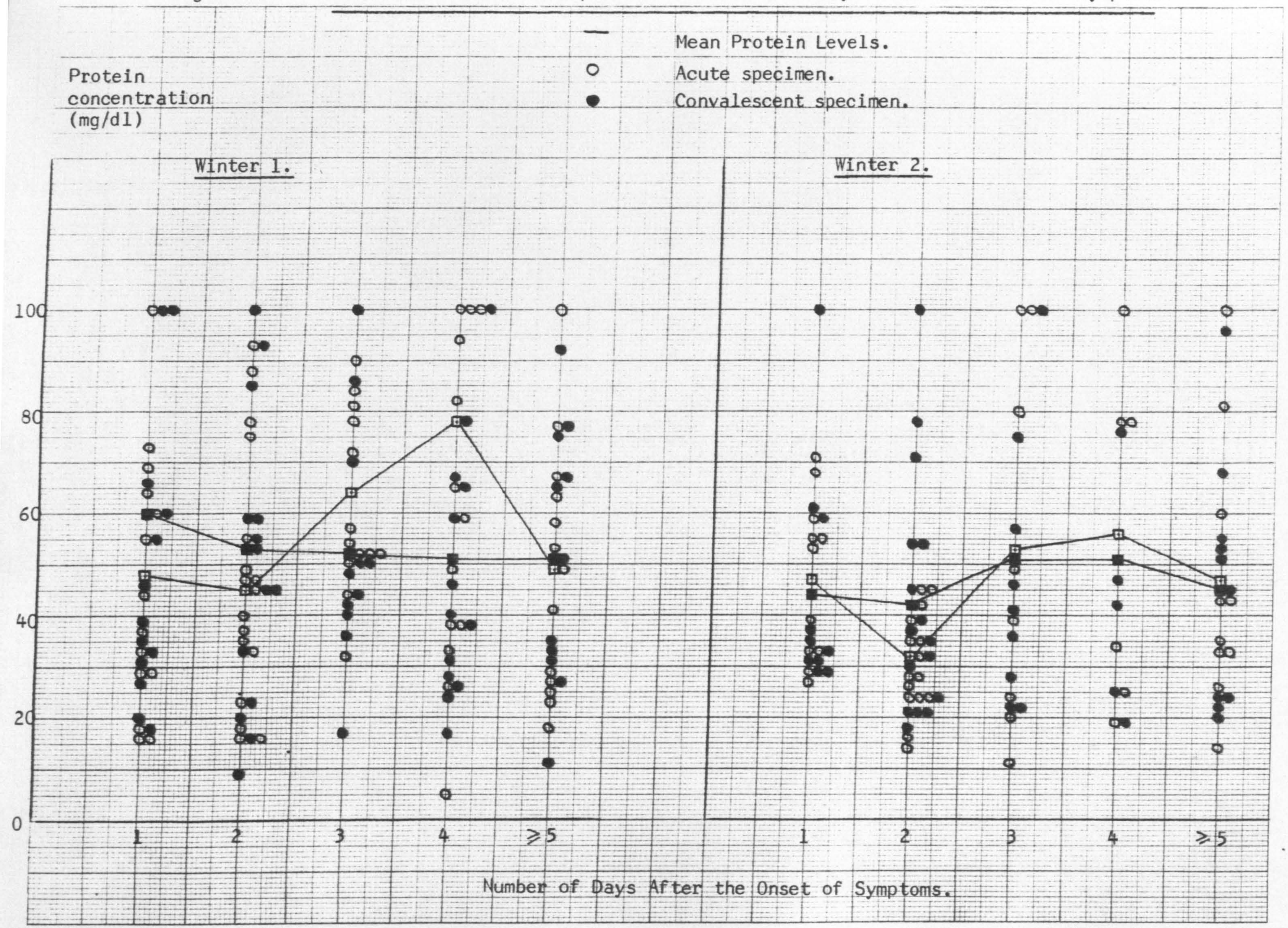


Figure 24. sIgA Production during Respiratory Infection.

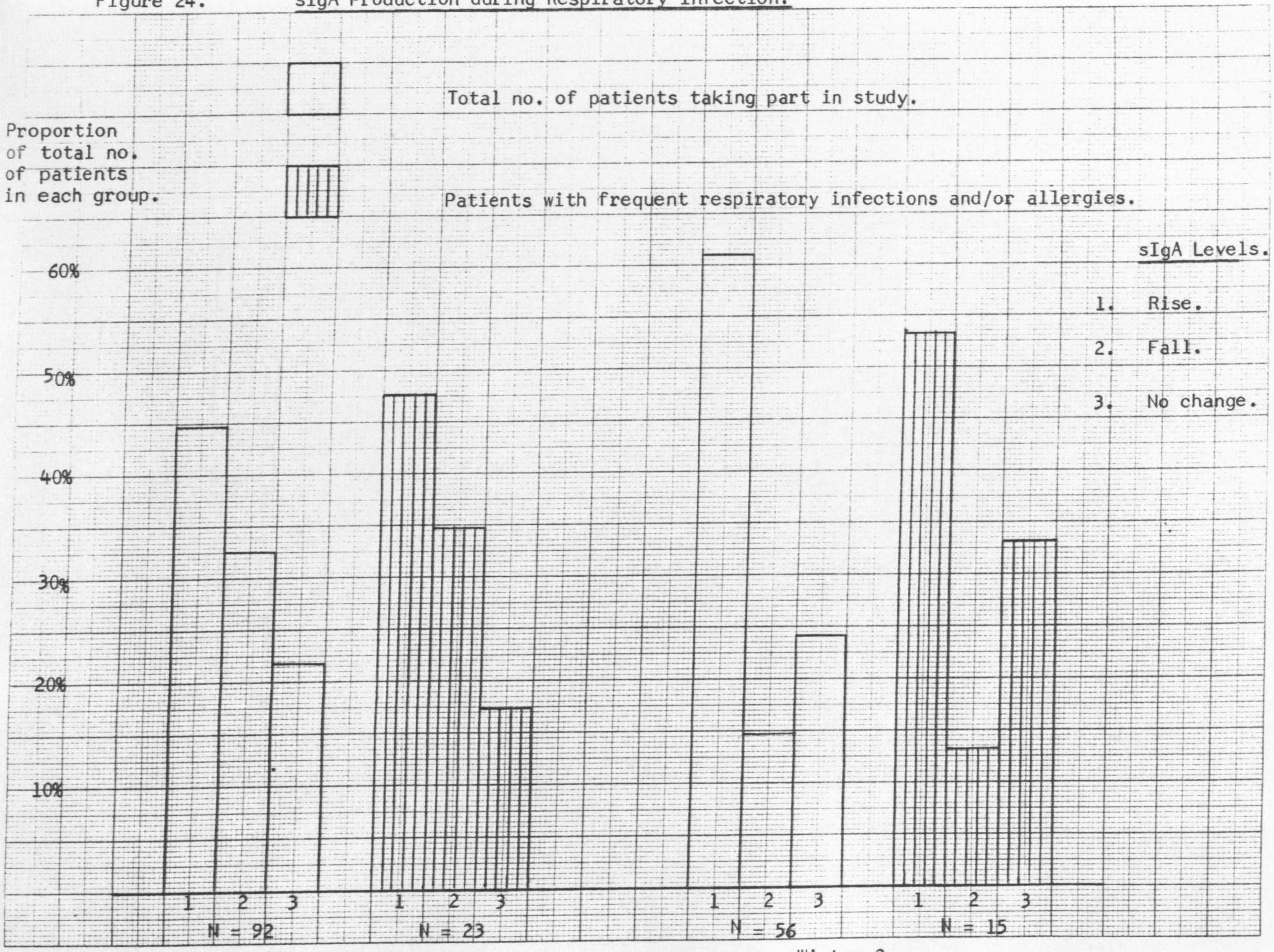
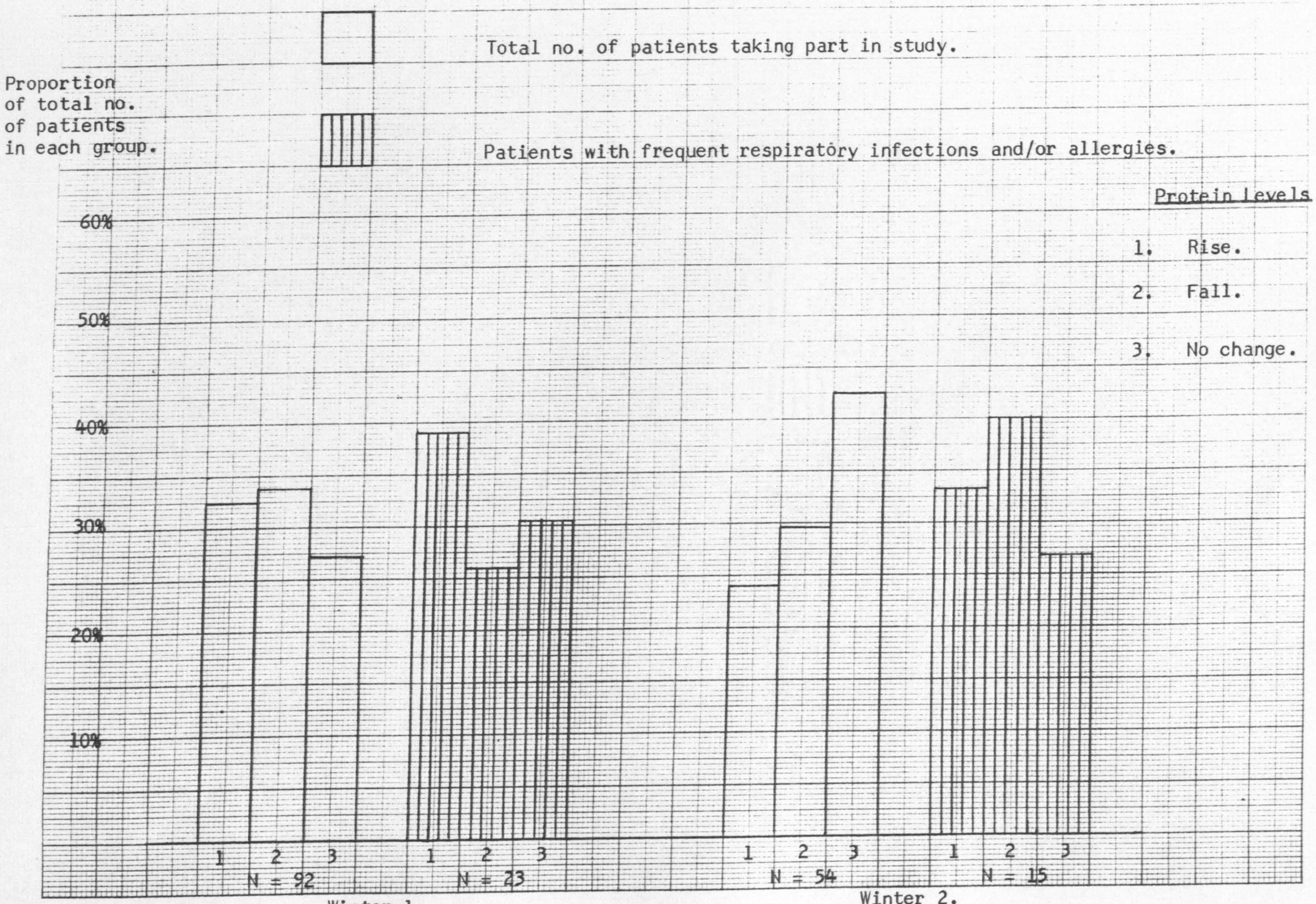


Figure 25. Protein Production during Respiratory Infection.



CHAPTER 4

DISCUSSION

Two important aspects of Influenza vaccination require further consideration. Firstly, which of the available vaccines is most efficacious and secondly, what is the duration of the immunity provided by the different vaccines?

Of the currently available vaccines, the main vaccine in routine use both in the United Kingdom and the United States is one in which the infectivity of the virus is destroyed chemically before the vaccine is injected subcutaneously or intramuscularly. Live virus vaccines made from attenuated strains remain experimental in most countries although they are used on a considerable scale in the U.S.S.R and Yugoslavia. Their merits relative to those of inactivated vaccine are still uncertain. A third possibility exists when inactivated vaccine is given as an intranasal spray. This method has been used in a number of Influenza vaccine trials in recent years and is under study in this thesis.

In the first part of this study an attempt has been made to evaluate the efficacy of an inactivated Influenza virus vaccine (Influvac Spray) administered intranasally by the third route i.e. by aerosol, and to assess the duration of protection induced. In addition,an evaluation has been made of the correlation between levels of local and serum antibodies and patient's response to subsequent artificial infection. A natural Influenza outbreak which occurred during the course of the study allowed some comparisons to be made of antibody responses to natural infection.

The long-accepted criterion for selection of volunteers to participate in vaccine trials has been the detection of very low levels of H-I antibody against Influenza virus. H-I antibody levels are not now thought to be the sole indication of protection and therefore can selection of volunteers on this basis still be justified? In the present study evidence has been presented to show how different initial levels of secretory antibody affect the response to the vaccine. The serological response in terms of H-I antibody will be examined first.

I have shown that subjects with low initial levels of secretory antibody, as measured by the H-I test, will develop a significant response following intranasal Spray vaccination (tables 4-6). If initial levels of secretory antibody are moderate to high the serological response to Spray vaccine is not significant. Secretory antibody is therefore necessary for protection.

In the group of subjects found to have moderate to high levels of secretory antibody before challenge (table 7) there are three who seroconverted after challenge (two in the Spray group and one in the placebo group). If immunity has been induced by the Spray vaccine why are there any seroconversions in this group? These conversions may have been due to the use of an attenuated challenge vaccine causing a 'boosting' effect of antibody production. Alternatively, the immunity conferred by the Spray vaccine has not lasted for the four month test period.

Subject 1723 (table 2) shows a four-fold rise in serum H-I antibody levels both six weeks post-vaccination (II) and four months later, pre-challenge (III). If thiswas due to natural infection why were no symptoms reported during the study ? It seems reasonable to suggest that the accompanying moderate to high levels of secretory antibody were reducing the severity of the clinical infection.

The presence of initial levels of neutralising antibody as measured by the Q-H assay do not prevent subjects producing a serological response to the Spray vaccine, but this response appears to protect against the attenuated challenge vaccine (tables 8-10).

If subjects have low levels of secretory antibody by both methods of measurement, there is a significant response to the Spray vaccine and no response to placebo. Unfortunately, in the group with moderate to high initial secretory antibody levels by both methods of measurement, there are too few subjects to analyse.

A small group of study subjects have levels of secretory antibody which varied according to the method of measurement. If Influenza neutralising antibodies, as measured by the Q-H test, are high and antibodies against the Haemagglutinin (HA), as measured by the H-I test are low, the subjects will respond to Spray vaccine and are immune to challenge; if antibodies against the HA are high and neutralising antibodies are low there is no response to Spray vaccine but a response does occur to the attenuated challenge vaccine.

The two tests must therefore be measuring different functional subgroups of secretory antibodies. If HA antibodies are high they appear to protect against the inactivated Influenza virus vaccine but this protection is short-lived and a response is elicited by the attenuated challenge vaccine. If neutralising antibodies are high the subjects can respond to the Spray vaccine but are immune to challenge. Neutralising antibodies do not give protection against the inactivated vaccine.

Why there should be a difference in the responses is unclear because neutralising antibody should, in theory, be directed against the Haemagglutinin (H.A.) and should therefore be comparable with H-I antibody. Neutralising antibody must also have a component which is active against the Neuraminidase (N.A.) antigen. This is also present on the virus surface.

In making this distinction it is important to realise that antibody measurement is dependent on the type of assay system used. Yanagihara and McIntosh (1980) found that local antibody to Respiratory Syncytial Virus (R.S.V.) and Parainfluenza viruses, post natural infection, was detected by the Fluorescent Antibody Test (F.A.T.) but not by neutralising antibody assays. This suggests a dissociation between functional antibody measured in the neutralisation test and antibody detected by assays for antigen-antibody binding. Murphy et al. (1982) showed that IgA antibodies measured by the Enzyme Linked Immunosorbent Assay (E.L.I.S.A.) correlate with neutralising antibody.

The present observation that antibody against H.A. must be present for protection confirms previous studies. Slepushkin et al (1971) showed that antibody against the H.A. was necessary for protection against the same Influenza serotype and that antibody against the N.A. does not protect. Schulman (1969) thought that anti N.A. antibody was important shortly after an epidemic in reducing the severity of infection and affecting transmission but less important thereafter in terms of protection.

In the present study the subjects who suffered from natural Influenza infection show a sustained rise in both serum and secretory antibodies (tables 2, 14, 18).
Since the time of infection was unknown it is not possible to comment on the rate of formation of the various antibodies.

In this section I have been dealing with the serological response to intranasal spray vaccination when an inactivated virus is used. The vaccine is eliciting a significant serological response and, in addition, it is clear that the initial levels of secretory antibody affect the response to the vaccine. This lends weight to the suggestion that initial levels of secretory antibody should also be used as a criterion for acceptability into vaccine trials.

The secretory responses to the inactivated Spray vaccine are puzzling. When they are measured by the H-I test, I have shown that, although a significant response is produced in patients in the Spray group, a comparable response is also produced in the placebo group (tables 14- 17). Of the subjects in the Spray group who show a four-fold rise in secretory antibody post Spray vaccine (II) all but one also show a four-fold serological rise. In addition, the secretory antibody rise is sustained for the four months of the test period. Of the subjects in the placebo group who show a four-fold rise in secretory antibody in the same period, none developed a comparable serological response and the rises in secretory antibody are not sustained for four months.

It seems that subjects who are vaccinated with Spray vaccine are producing a genuine response whereas those vaccinated with a placebo may only be responding to the irritation of intranasal aerosol treatment which, in turn, promotes both the non-specific activation of the secretory immune response and mucus production. As all the subjects are adults it is highly likely that they will have been 'primed' against Influenza type A (H3N2) strains which have been active since the pandemic of 1968 and it is fortuitous that specific antibody is being produced.

It is also reasonable to suggest that the subjects who have responded in the placebo group are reflecting the fact that Influenza virus is present in the community. It has been suggested recently by several workers (Ganguly and Waldman (1977), Schvartsman and Grigorieva (1979)) that secretory immunity is more important in protecting against the different strains of Influenza virus (drifts) than in protection against new variants (shifts). In this latter case the serological response is more important. The placebo group response may well represent a 'boosting' of herd immunity.

Evidence for IgA memory has been published recently. (Wright et al. (1983)). These authors found that live, attenuated Influenza vaccine administered 10 to 18 months before challenge with inactivated vaccine clearly primed the secretory immune system for local memory and caused an 'anamnestic' response on re-exposure. Neither serum nor local antibodywas seen before two weeks from primary exposure. Local response was demonstrated however at one week after re-exposure.

This postulated combined effect of systemic and local immunity emphasises the importance for current vaccines to stimulate both systems. Clearly in inter-pandemic years the more important antibody response to stimulate is the secretory response.

A higher percentage of the Spray group (35%) compared with the placebo group (19%) have moderate to high initial levels of secretory antibody (figure 5). Since the presence of secretory antibody protects against both vaccine and challenge, this factor may cause the Spray vaccine to appear less effective.

When the secretory responses (measured by the Q.H. test) are examined (tables 18- 21) the overall response is smaller than that demonstrated by the H-I test. The pattern is similar however and the above reasons also apply.

The duration of complete resistance to Influenza A in the absence of antigenic shift and following recovery from the natural disease is unknown. It is believed to last only two years (Lennette (1981)). Some workers, however, indicate that immunity persists for at least twice this time and that significant resistance to Influenza A virus may last for at least 21 years (Chanock and Murphy (1979)). Because of the uncertainties which still surround the duration of naturally acquired immunity, frequent vaccination is generally advised. In theory, at least, mass immunisation against Influenza should serve either to prevent epidemics or to halt their speed of spread. The phenomena of antigenic drift and shift require that immunisation be against those viral strains that are currently in circulation in the population at risk. For this reason the antigenic composition of vaccines must be constantly under review.

In this study the duration of Spray vaccine induced immunity was examined for four months (ie. over the peak period of exposure to Influenza virus).

Figure 5 shows the total and specific mean secretory antibody titres during the four month test period for the Spray vaccine, placebo and naturally infected groups. The naturally infected group show a sustained rise in local antibody whereas the vaccine and placebo groups show a drop in titre after an initial rise over the four month period. Duration of the secretory antibody response in nasal washings was therefore higher after natural infection than following vaccination. In 1979, Schvartsman . and Grigorieva showed

that the duration of the secretory antibody response was higher after severe forms than after mild forms of Influenza. Concentrations of secretory antibodies (ie. 1: 4 by the Indirect Haemagglutination test (I.H.T.)), which in volunteers had been correlated with the decrease in virus isolation in nasal secretions, persisted for four to eight months in both adults and children who had mild courses of disease and for more than eight months in persons who had severe forms of the infection.

The present study in which Influvac Spray, intranasal, inactivated vaccine was used is a 'follow-up' study to an earlier one carried out in Rome (Chezzi et al. (1976) unpublished). The strain of virus used in the vaccine was Influenza A/England/42/72 (H3N2). The results of the Rome placebo-controlled comparative study,which involved 157 volunteers, on the local and systemic antibody responses after inoculation of inactivated Influenza vaccine administered either by the inhalation or subcutaneous route are as follows: Administration of vaccine by inhalation (Influvac Spray) significantly increased the levels of neutralising antibodies but had no effect on the level of serum antibodies. On the other hand, inoculation of a similar vaccine (Influvac Plain) at a slightly higher dose by the subcutaneous route, stimulated circulating H-I antibodies but not neutralising local antibodies.

These findings contrast with the present study where serological and local responses occur. A different strain of Influenza was used in this study. (Influenza A/Victoria/ 3/75 (H3N2)). Also, placebo and Spray were administered intranasally whereas in the Rome study a parenterally administered vaccine was being tested at the same time (Influvac Plain) and the placebo was also administered parenterally. A third difference was that initial levels of secretory antibody were lower in the placebo group than in the Spray group in the Rome trial. The reverse is true in the present trial.

In the analysis of the results of Vaccine trials it is important to know why an effective or ineffective result was obtained. (Couch, R.B. cited in Kilbourne et al. (1973)). Like must be compared with like or incorrect conclusions may be reached. For example, in a study carried out in volunteers by Couch, one group received inactivated vaccine intranasally and the other received the vaccine intramuscularly. When the volunteers were subsequently challenged with infectious virus, the results in neither vaccinated group were significantly different from those in control groups. However, when formation of antibody after vaccination was assessed, it was noted that not all individuals responded to the vaccination procedure with formation of antibody. When only those who actually developed antibody were compared with controls, significant protection was noted in both vaccinated groups. In addition, both vaccinated groups developed approximately equal antibody responses in serum and nasal secretions, and the degree of protection was approximately the same. Had the antibody response not been assessed in this way, the conclusion would have been that vaccines given by any route are ineffective in producing protection, whereas in reality, the immune response to the particular preparation used and the method of vaccination was less than optimal. These considerations suggest that vaccine failures may be attributed frequently to failure of a vaccine preparation or method of vaccination to elicit an immune response rather than to failure of an elicited immune response to protect against disease.

There is ample recorded evidence attesting to the protective capacity of killed Influenza virus vaccines. Their protective efficacy averages 70- 90% and is sufficiently high to find practical application in Public Health. Such application, however, has been directed primarily at selected population groups, in particular the elderly and other individuals who are immunologically compromised or suffering from chronic or debilitating disease. High priority is also

accorded to key industrial workers in an effort to reduce absenteeism and the attendant losses in productivity. (Lennette (1981)). Because immunity following vaccination is of uncertain duration and since Influenza type A viruses are undergoing continuous antigenic modification, re-vaccination on an annual basis is recommended.

Inactivated whole-virus vaccines administered parenterally give rise to a low incidence of local or systemic reactions. Because of these, children, who form an important part of the population at risk, have generally been excluded from immunisation programmes. However, increased realisation that Influenza A virus is a cause of serious disease in infants and young children has led to increased research into alternative methods of administration of vaccine. (Glezen et al. (1977)).

Resistance to Influenza represents a complex and subtle interaction of humoral and cellular factors. In consequence, a more effective state of resistance might be achieved through the use of a live, attenuated virus vaccine which mimics natural infection more closely than the immunity induced by inactivated virus vaccine.

Conventionally, live, attenuated vaccines are administered to the upper respiratory tract by the intranasal route. In adults and children these vaccines have been shown to be highly immunogenic with an acceptable rate of reactogenicity (Tyrrell et al. (1981)). However, the major fear of attenuated vaccines is their possible reversion to virulence in an unprotected population because of the risk of genetic recombination.

Another curious fact which has emerged from recent studies is that mammalian cells, infected by Influenza type A (as well as by other viruses) show an increased binding of pathogenic bacteria and an increased incidence of bacterial

superinfection. It has been suggested that the use of attenuated vaccines in children may lead to an increased incidence of bacterial upper respiratory tract infections. For example, otitis media and/or pharyngitis may be more common in these situations and these two diseases should probably be considered as potential vaccine complications. These complications may not yet be significant in the United Kingdom or United States but are of potential significance in countries such as Japan or the U.S.S.R. where the major emphasis of Influenza immunisation is in children and where attenuated Influenza virus vaccines are currently in use. (Kessler (1980)).

Meanwhile, for as long as inactivated Influenza virus vaccines are in use in this country, it will be necessary to keep trying to complete the picture of anti-Influenza immunity both local and systemic so that current vaccines can be made more effective

In the second part of this thesis an attempt has been made to study secretory immunity following naturally acquired Influenza and other respiratory bacterial and viral diseases. The dynamics of secretory antibody production have been investigated in terms of time of initiation, variation in total duration and quantity of production during infection with different recognised bacterial and viral pathogens. Particular attention has been given to the patient's previous history of respiratory infection and to the possibility of malfunctions in the secretory system as may happen in chronic bronchitis and emphysema.

The initial results in this section have confirmed that there is a significant response in terms of secretory IgA production following natural infection with a bacterial or viral respiratory pathogen. (table 30).

It should be noted that in Winter 1 there were five patients with no detectable sIgA. The possibility arises that these patients may have been IgA deficient.

Serum IgA deficiency is commonly defined as an IgA level below 0.02mg/ml. (being the detection limit of the S.R.I.D.). When the concentrations of IgG and IgM in the serum are normal or increased, the designation ' selective IgA deficiency' is used. Selective IgA deficiency can occur without clinical manifestations : in patients the symptoms are mainly those of gastrointestinal and/or respiratory infections. Since serum IgA is mostly (but not always) associated with secretory IgA deficiency, the clinical symptoms reflect the latter deficiency rather than the former. The incidence of selective IgA deficiency in a normal population has been estimated to be between 0.03 to 0.28% and an occurrence of various autoimmune phenomena in these individuals is well known.

In addition,the occurence of transient IgA deficiency as well as partial IgA deficiency occurs. Transient IgA deficiency has been observed in children who were slow to form serum IgA, or following tonsillectomy or following the use of certain drugs such as penicillamine and phenytoin. Slow maturation of IgA synthesis early in life may lead to the development of allergic disease.(Review of IgA Defiency, Schuurman and Zegers (1980)).

Three of the five patients with no detectable IgA had normal levels of serum immunoglobulins (the other two patients could not be traced.) This suggests that they are unlikely to be IgA deficient. In addition, three patients gave no history of frequent respiratory infections and/or allergies. In one the history was unknown; the fifth patient is subject to allergic rhinitis.

In all but one patient the levels of protein detected in the nasal swabs were normal. This excluded incorrect sampling. The fifth patient's sample had a very low protein content, presumably because only a small amount of mucus had been collected instead of the correct sample. It is also possible that a highly mucoid sample might 'trap' the sIgA and render it undetectable in the electrophoresis gel system.

In several specimens the sIgA concentration fell to zero during the seven day interval between specimens. This is highly unlikely to happen and these specimens must be viewed with suspicion. It is possible that the sIgA is being bound by the replicating pathogen and is therefore not free to react in the detection system. (Ogra and Karzon (1971)). Alternatively, sIgA may have fallen to a level below the sensitivity if the electrophoresis test. These possible explanations for falls in sIgA concentration are more likely to be correct when a live pathogen is replicating than when an inactivated vaccine is administered.

Variation in mucus production occurring during the course of a respiratory infection will also affect the amount of sIgA detectable.

Bacterial pathogens were isolated equally often from either 'acute' or 'convalescent' specimens whereas all but two of the viral pathogens were isolated from the 'acute' phase specimen.

In a recent article in The Lancet (Editorial, February 27th., 1982) the possibility that viruses may impair the immune response against bacteria is discussed. As well as damaging bronchial and alveolar epithelium, Influenza viruses often interfere with chemotaxis of polymorphs and macrophages and probably also depress immune T-cell function. In addition, there is the previously mentioned problem that infection with haemagglutinating viruses makes mammalian cells show an

increased bacterial adhesion which results in non-specific binding with increased susceptibility to bacterial super-infection. Adenovirus infections, for example, are seldom complicated by bacterial pneumonia.

Certain micro-organisms of importance in human disease such as Neisseria sp., Candida sp. and Vibrio sp. have been shown to produce proteolytic enzymes whose only known substrate is human IgA. These IgA proteases impair the antibody function of IgA even though these enzymes do not directly affect the primary structure of the antigen-binding (F-ab) region of the molecule. Such interference with the local defence mechanisms is likely to play an important role in pathogenesis. (Plaut (1978)).

Bacterial superinfection may therefore be a reason for the higher incidence of 'convalescent' phase bacterial isolates. Local defence mechanisms may be impaired for the reasons given above. Interferon production may also be important.

The 'initial' level of sIgA depends on several factors. The duration of the'latent period' of secretory antibody processing and accumulation, the level of sensitisation of the secretory immune system to the pathogen and the intensity of development of the infectious process (ie. the length of time after the onset of infection) are all important.

During the first few days after the onset of respiratory infection the accumulation of sIgA in secretions is higher in patients with low initial levels of sIgA. This suggests that the build-up in concentration of antibody may be happening early in patients, just after the first clinical symptoms appear.

The suggestion confirms the findings of Hruscova et al (1976) but contrasts with those of Schvartsman and Grigorieva (1979) and Rossen et al. (1970) who showed that the duration of the latent period of sIgA production in Influenza A varied from 3 - 21 days and in some cases was even longer.

Rapid build-up in concentration of sIgA may be attributed to factors such as the problems involved in pinpointing the time of onset of infection. Unlike the administration of vaccine the time of onset of natural infection is arrived at as an assessment between patients and doctor. It is important to realise that the time may be several days before the onset of symptoms, also the size of the infecting dose is unknown and this too affects sIgA production. (Schvartsman and Zykov. (1976)).

Only a small amount of IgA is present in the sera of newborn babies (Tomasi and Gray (1972))and in a 6 month old child levels of serum IgA are equal to only 25% of the amount formed in a healthy adult. (Uffelman et al (1970)). However, in the child sIgA reaches adult levels by the end of the first month. In this study,during both winter periods,children under the age of 4 years showed a significant sIgA response to respiratory infection and appeared to be synthesising antibodies more actively than adults. This finding confirms reports from a recent Influenza workshop (Kilbourne et al (1973)) but contradicts observations by Waldman et al, 1971 who suggests that secretory antibodies are not formed as actively in small children as in adults apparently due to what the authors described as children's less intensive production of sIgA.

The duration of antibody production following natural infection was studied in 14 patients. Each showed an individual pattern of sIgA production. This individual response caused difficulty in assessing the duration of the response because no clear pattern emerged.

In some patients detectable sIgA was maintained for up to 12 months; in others the levels dropped but were re-stimulated rapidly following re-infection.

Wright et al (1983) have shown that secretory antibody formed after natural infection with Influenza is rather persistent. Antibody was observed in 8/8 children for 3 - 6 months post primary infection.

When the patients in this study were examined as a group it became clear that although levels of sIgA were falling,they were maintained at a rocket height of 3 mm for at least 9 months. By the 10th month, presumably, the respiratory pathogens were again becoming active in the community and secretory immunity was boosted. Total protein production followed a similar pattern.

Information about the influence of respiratory infectious diseases on the formation of secretory antibodies is scanty and often contradictory. In adults and children who are ill with different forms of bronchial asthma, secretory antibodies are formed in response to the aerosol administration of inactivated Influenza vaccine exactly as in healthy people (NewcombandEller(1971),Waldman et al.(1971)). On the other hand, patients with bronchopulmonary disorders had weak reactions to the same vaccine. It is possible that bronchitis lessens the intensity of antigen sorption and therefore also lessens the intensity of 'trigger' action. (Shore et al.(1973)). South et al, (1968) have shown that in infants a correlation can be demonstrated between the absence of sIgA and repeated episodes of acute and susceptibility to chronic respiratory tract infections, regardless of levels of serum immunoglobulins.

In this study,patients with a history of repeated respiratory infections and/or allergies showed a similar sIgA response to the study group as a whole (ie. no deficiencies in sIgA production were found).

The sIgA and total protein variations are remarkably similar over both winter periods. In addition,no correlation was observed between base-line levels of sIgA and the frequency of respiratory infections. This confirms the work of Yodfat and Silivan(1977).They found that children who responded to infection by producing higher levels of sIgA had a lower incidence of respiratory tract infection than those who did not. This finding,in turn,supports the hypothesis that the responsiveness of the host in the production os sIgA during infection is important in protection against mucosal infections.

CHAPTER 5

AREAS FOR FUTURE DEVELOPMENTS

Recent technological advances have brought new, rapid antibody-detecting tests to the laboratory. Sensitive tests such as F.A.T. (Fluorescent Antibody Test), E.L.I.S.A. (Enzyme-Linked Immunosorbent Assay) and R.I.A. (Radio-immunoassay) have great implications in vaccine research. sIgA testing is no longer a laborious, time-consuming problem and large numbers of secretions can be screened quickly and easily.

The detection and quantitation of specific sIgA also has applications in the Diagnostic Laboratory. Using E.L.I.S.A., Grauballe et al. (1981), have shown that Rotavirus infections can be diagnosed by finding specific sIgA in serum. The detection and quantitation of specific sIgA in serum and in secretions for other viruses will require evaluation but, in time, will surely become an accepted method for diagnosis of virus infections.

sIgA is important in protection from infection but its role in recovery is still unclear. sIgA production during recovery is part of a more complex immunological process and is subject to extensive variations within a group of individuals. The importance of T-cell immunity in recovery from Influenza has only been recognised recently. (Wells et al. (1981 a & b); Yap et al. (1978)). The mechanism of action of the T-cells is not fully understood. However, they also are obviously only a part of a very complex immune system for they initiate the antibody response by priming B -lymphocytes and they also act independently. T-cell immunity on its own does not confer complete immunity but is likely to be part of the recovery process.

During the same time period as the vaccine trial presented in this thesis (1977 - 78), large scale clinical trials were being undertaken in USA to determine the safety and optimum dosage of inactivated influenza virus vaccine administered parenterally. The results of these trials demonstrated that the test vaccines were safe and that optimum serological responses could be achieved when the vaccines were standardised with respect to their haemagglutinin content. (Galasso et al (1977)). In 1979, four different inactivated influenza virus vaccines were tested for their reactogenicity, immunogenicity and for the subsequent persistence of induced antibody in adults. The results, confirmed the American studies but still left unanswered questions about the degree and duration of protection offered by the various vaccine schedules. (Cate et al (1983)).

Inactivated influenza virus vaccines are usually administered subcutaneously. Given this way they can elicit both local and serum antibody responses (Dowdle (1973)). Their use by the intranasal route has not received much support presumably because their effectiveness by this route has not been shown to be superior.

Despite improvements such as the development of 'subunit' influenza vaccines the protection conferred is not complete. As a consequence there is renewed interest in live, attenuated vaccines which would mimic natural infection in its 'broader' and more durable immunity. Murphy et al (1982) and Wright et al (1983) have shown recently that stimulation of secretory immunity by the use of live virus is important in protection following influenza vaccination.

Only when the total complexity of the immune response to influenza is elucidated will it be possible to devise completely effective vaccines. The eradication of respiratory disease will then be a realistic goal.

APPENDIX I

Contractions used in this Thesis.

Ab.	:	Antibody
Ag.	:	Antigen
AgMK	:	African Green Monkey Kidney
B.A.L.T.	:	Bronchus Associated Lymphoid Tissue
C.D.S.	:	Communicable Diseases Scotland, Ruchill Hospital, Glasgow.
C.F.	:	Complement Fixation
d or x	:	Mean
d. of f.	:	Degrees of Freedom
E.I.D.50	:	Egg Infective Dose (50%) ie. the dose of virus which infects 50% of the eggs under test.
E.L.I.S.A.	:	Enzyme Linked Immunosorbent Assay
F.A.T.	:	Fluorescent Antibody Test
F.I.T.C.	:	Fluorescein Isothiocyanate
F (ab)	:	Antigen binding region of immunoglobulin
F (c)	:	Complement fixing region of immunoglobulin
G.A.L.T.	:	Gut Associated Lymphoid Tissue
G.I. tract	:	Gastro-intestinal tract
G.U. tract	:	Genito-urinary tract
G.M.T.	:	Geometric Mean Titre
ɣ-globulin	:	Immunoglobulin
H.A.	:	Haemagglutinin
H.A. unit	:	Haemagglutinating unit
H-I	:	Haemagglutination-Inhibition
IgA	:	Immunoglobulin type A
IgE	:	" type E
IgG	:	" type G
IgM	:	" type M
I.D.D.	:	Immuno Double Diffusion
I.E.	:	Immunoelectrophoresis
Infs.	:	Infections
I.U.	:	International Units

L	:	Lymphocyte
M.E.M.	:	Minimal Essential Medium
N	:	Population number
N.A.	:	Neuraminidase
O.D.	:	Optical Density
P.B.S.	:	Phosphate Buffered Saline
P.P.	:	Peyer's Patches
Q.H.	:	Quantitative Haemadsorption
r.b.c.	:	Red blood cells
R.D.E.	:	Receptor Destroying Enzyme
R.I.A.	:	Radioimmunoassay
S.C.	:	Secretory Component
S.D.	:	Standard Deviation
S.E.	:	Standard Error
sIgA	:	Secretory immunoglobulin type A
S.R.D.	:	Single Radial Diffusion
S.R.H.	:	Single Radial Haemolysis
U.R.T.I.	:	Upper Respiratory Tract Infection

APPENDIX II

PROTOCOL FOR A DOUBLE-BLIND PLACEBO-CONTROLLED STUDY OF LOCAL AND SERUM ANTIBODY RESPONSES OVER A PERIOD OF FOUR MONTHS AFTER INTRANASAL ADMINISTRATION OF INACTIVATED INFLUENZA VIRUS VACCINE AND OF THE IMMUNITY CONFERRED AS ASSESSED BY ARTIFICIAL INFECTION WITH AN ATTENUATED LIVE VIRUS VACCINE STRAIN.

Product	:	Influvac Spray
Kind of Study	:	1. Antibody response (local and serum) over 4 months 2. Protection against challenge with Gripovax RIT 4050
Country	:	United Kingdom
Route of Administration	:	Intranasal (Influvac Spray and Gripovax)
Dosage	:	Influvac Spray : 400 I.U. A/Victoria/3/75 (H3N2) 400 I.U. B/Hong Kong/8/73
	:	Gripovax : 10^7 EID_{50}
No. of Subjects	:	about 40 pre-selected from 150.

Composition of Vaccines.

The antigenic composition of the vaccines used in this study will be as follows per dose :

1.	Influvac Spray	: A/Victoria/3/75 (H3N2)	400 I.U.
		B/Hong Kong/8/73	400 I.U.
		1 dose = 4 "puffs" intranasally.	
2.	Gripovax	: A/Victoria/3/75 (H3N2) strain RIT4050	
		1 dose = 5 drops of reconstituted vaccine into each nostril. (10^7 EID_{50}).	
3.	Placebo material	: an aerosol with the same appearance and composition as Influvac Spray except that it does not contain virus.	

The above vaccines are registered in the United Kingdom and are approved by the National Institute for Biological Standards and Control.

Selection of Study Subjects.

About 150 previously unvaccinated healthy subjects will be initially invited by an explanatory circular letter to participate in the investigation. They will be informed of the need to collect blood samples and nasal washings on several occasions during the course of the study which will cover a period of about 6 months. Volunteers will be told that an investigation of the efficiency of an inactivated spray vaccine will take place during the vaccination programme which will also involve inoculation of a live attenuated Influenza vaccine. An outline of the study will be given. They will be asked to sign a consent form. Volunteers will of course be completely free to withdraw from the study at any time.

Excluded from this Study will be :

- all subjects who are known to be hypersensitive to :
  - egg products, chicken meat or feathers
  - neomycin
- all subjects who at the time of the vaccination show symptoms of acute or chronic respiratory disease
- all subjects who show pre-vaccination serum H-I titres >40 against Influenza A/Victoria/3/75.

Allocation to Study Groups and Vaccination Scheme.

Subjects included in the study will be divided at random in 2 equal groups according to a pre-determined scheme. It is hoped that at least 40 subjects (ie. 20 subjects per group) will be recruited.
Vaccination with a freeze-dried, bivalent, aerosol, inactivated Influenza vaccine (Influvac Spray) and a placebo preparation will be done on a double-blind basis.
Four months after vaccination all subjects will receive one dose of a live monovalent vaccine (ie. A/Victoria/3/75 (H3N2) : Gripovax).

Mode of Administration of Vaccines.

Influvac Spray and the spray placebo will be administered into both nostrils during deep and slow inhalation. A single dose of the vaccine (ie. 4 "puffs", 2 into each nostril) will be administered.

Gripovax will be administered by instilling 5 drops of the reconstituted vaccine into each nostril with the subject lying supine with head extended and eyes closed (as recommended by the manufacturer).

Collection of Specimens.

A. Blood Sampling

Blood (I)

From all volunteers (potential participants) blood samples (8 ml.) will be collected before (on Day -14) the start of the study (vaccination on Day 0).

Blood (II)

From all study participants, a second blood sample (8 ml.) will be collected 6 weeks after vaccination (on Day 42).

Blood (III)

Before administration of live vaccine (on Day 112) a new blood sample (8 ml.) will be taken from all subjects.

Blood (IV)

From all participants a final blood (8 ml.) sample will be taken 6 weeks after vaccination with live vaccine.

B. Nasal Washings

These will be collected from all subjects on the day of vaccination (1/2 hour before) and subsequently 6 and 16 weeks after vaccination and again 6 weeks after inoculation of the live Influenza vaccine.

Labelling of Samples

The blood samples and nasal washings should be labelled with the white labels supplied by Duphar.
On the label should be clearly mentioned I, II, III or IV with the date of taking the sample and the code number of the subject.

Storage and Transport of Serum.

Separation of serum from the collected blood samples should be performed on the day of collection. The first batch of sera (about 150) will be titrated for H-I antibodies against A/Victoria/3/75 (H3N2). After titrations, the remaining sera will be stored at -20 C. The set of sera from the second, third and fourth blood collected will be stored at -20 C until used.

Titration of Sera.

This will be done by the standard Haemagglutination-Inhibition (H-I) titration techniques in routine use in Belvidere Hospital Laboratory. The first batch of sera will be titrated directly on arrival in order to select those subjects who will be admitted to the study. At the end of the study all collected sera from each subject will be titrated simultaneously, using the vaccine strains as antigens.

Titration of Nasal Washings.

All nasal washings will be titrated at the end of the study for total and specific sIgA by the techniques in current use in the Belvidere Hospital Laboratory. Until used they will be stored in the frozen state.

Material and Coding.

Influvac Spray and the placebo material will be prepared by Philips-Duphar B.V. and provided in code numbered vials of identical appearance.

The coding will be done by the Statistical Department of Philips-Duphar B.V.

The live attenuated vaccine (Gripovax) will be imported from Belgium.

Evaluation of Results.

Infection with the live vaccine will be diagnosed by serology. A 4-fold or greater rise in serum H-I and/or C.F. titre against A/Victoria/3/75 and/or a significant local specific antibody response will be taken as diagnostic.

Safety Precautions.

It is important that all Physicians involved in this study follow the protocol exactly and remember that some of the exclusions in the patient selection scheme are designed to avoid inclusion of patients who may react adversely to one or more agents administered during the study or to inclusion in a clinical study of this type.
Very few therapeutic agents are completely devoid of all possible risks of causing side effects. The Physicians involved in the execution of this study should adhere to the protocol and should be aware of what are the main possible side effects of the drugs used in this study and the investigator should ensure that any drugs or equipment required to handle such situations are reasonably accessible during the study.

Date of Start of Study.

October, 1977 (Ist. blood sample).

End of Study.

April, 1978.

It is important that the investigator and his trained colleagues, whom he selects to assist him in this study, adhere strictly to this protocol.

Table 39. Summary of Vaccine Scheme and Collection of Specimens for Laboratory Investigation.

Procedure	Stage of Study				
	Day -14	Day 0	Day 42	Day 112	Day 154
Blood sampling	+		+	+	+
Nasal washing		+	+	+	+
Vaccination		+		+	
Number of subjects	± 150	40	~ 40	~ 40	~ 40

APPENDIX III

Statistical Analyses

1. Geometric Mean Titres

The Geometric mean is obtained by multiplying all the values together and taking the $N^{th.}$ root of the product where there are N values in the sample.

This may be expressed

$$g = \sqrt[N]{P\ (x)} = \sqrt[N]{x_1 \cdot x_2 \cdot x_3 \cdot \ldots \cdot x_N}$$

where g is the geometric mean and P (x) is the product of all the values of x. It is most easily calculated by using logarithms. If the logarithms of all the numbers are added together, the sum of the logarithms will be the logarithm of the product of the numbers. If this sum is divided by N, it will give the logarithm of the Nth. root of the product, that is the logarithm of the geometric mean. Therefore the antilog of this mean value is the Geometric mean value.

2. Coefficient of Variance.. ($C.V. = \frac{S.D.}{\bar{x}} \times 100\%$)

The coefficient of variance is a percentage of the standard deviation divided by the mean of a set of data. It is a measure of the variability of the data.

3. The Student 't' Test.

The 't' test was used to determine whether there was a significant difference between the values found in two discrete sets of data. This analysis compares the means and standard deviations obtained from both populations using the following formula :

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{SD_1^2}{N_1} + \frac{SD_2^2}{N_2}}}$$

where $\bar{x}$ = mean
SD = standard deviation
N = population size

The 't' value is then compared with the 't' distribution table. (Fisher and Yates (1963)) at $(n_1-1) + (n_2-1)$ degrees of freedom and a P value is obtained which indicates the probability that two sets of data are related, assuming the normal distribution. A significant difference is one which has a probability of less than or equal to 5% which corresponds to 1.96 times the standard error. If the probability is less than or equal to 1%, the difference is highly significant.

APPENDIX IV

Buffers and Reagents.

1. Buffers :

Phosphate Buffered Saline

8.0g	Sodium chloride
0.2g	Potassium dihydrogen orthophosphate
2.9g	Di-sodium hydrogen orthophosphate
0.2g	Potassium chloride
1 Litre	Distilled water.

Michaelis' Buffer (for Immunoelectrophoresis)

19.43g	Sodium acetate	Solution A
29.43g	Barbital sodium	
1 Litre	Distilled water	
8.5%	Sodium chloride solution	Solution B
0.1N	Hydrochloride solution	Solution C

Add 50ml. of solution A to 20ml. of solution B. Adjust pH. to 8.6 with solution C. Make up volume to 250ml. with distilled water.

2. Media :

Monkey Kidney Medium

(for the growth and maintenance of monkey kidney cells)

M.E.M. medium and Earles Salts containing

0.85g/litre	Sodium bicarbonate	
10%	Foetal Bovine Serum	(Growth medium)
2%		(Maintenance medium)
100 units/ml.	Penicillin	
10ug/ml.	Streptomycin	
25 units/ml.	Nystatin	
300ug/ml.	Glutamine	

Monkey Kidney Serum-Free Medium

(for Quantitative Haemadsorption Test)

199 medium and Earles Salts containing

2%	Hepes
0.15%	Sodium bicarbonate
100 units/ml.	Penicillin
10ug/ml.	Streptomycin
25 units/ml.	Nystatin
300ug/ml.	Glutamine

3. Immunoelectrophoresis Reagents :

Coomassie Stainer

5g	Coomassie Brilliant Blue
450ml.	Ethanol (96%)
100ml.	Acetic acid
450ml.	Deionised water

Add Coomassie dye to Ethanol/ Acetic acid mixture and leave at room temperature overnight. Filter the solution before adding the water.

Destainer

250ml.	Ethanol (96%)
100ml.	Acetic acid
450ml.	Deionised water.

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ADDENDUM

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